

Medical Parasitology in the Philippines

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Vicente Y. Belizario, Jr.
Winifreda U. de Leon
EDITORS



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The data in this book have been verified with reliable sources, and treatment modalities suggested have been utilized in clinical practice. However, new researches and changes in the medical sciences should be considered. Readers are advised to consult other sources such as drug information sheets and dosage, contraindications to administration, and other relevant data.

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*To our fellow Filipinos,
from whom we derive inspiration and learning,
especially those who are poor and neglected,
suffering from the burden of parasitic diseases*

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Foreword

No other book published by the University of the Philippines Manila (UPM) has been as widely patronized both by UPM constituents and other health students and professionals throughout the country than the Philippine Textbook on Medical Parasitology, now entitled Medical Parasitology in the Philippines.

That the response to the first two editions of the book has been overwhelming affirms the value and significance of the material in complementing meager publications on medical parasites with special focus on the local setting.

Dr. Vicente Y. Belizario, Jr. and the contributors of the book for both editions deserve commendation for responding to the need for a locally compiled comprehensive material on parasitology through their painstaking work on this book.

It is good to know that the book that first came out in 1998 has been updated again through this third edition. The additional data and information, fresh insights, and new experiences shared by the authors at the global, regional, and national settings, make

the book all the more relevant to policy makers, practitioners, students, and health workers involved in eradicating parasitism in highly affected communities.

To this day, parasitic infections are still considered a major public health problem in the Philippines and the rest of the Asian region. For a developing and tropical country like the Philippines, the prevalence of parasitic diseases is worsened by high population density, hot and humid climate and other environmental factors, poverty, and socioeconomic conditions that provide a conducive setting to the parasites.

Notwithstanding the difficulties and struggles of fighting parasitism, all sectors should come together and join efforts to combat the disease because of its grave effects on the health, productivity, and well-being of the people.

I am confident that his latest edition of the book will serve as an accurate and valuable reference material in the continuing war against parasites.

Thank you again for this gem of a textbook.

MANUEL B. AGULTO

Chancellor

University of the Philippines Manila

Foreword to the Second Edition

The preparation of this Philippine Textbook of Medical Parasitology merits commendation for many reasons. It is a precious product of collaborative effort among the top parasitologists in the country, including faculty members from different medical and science colleges. The comprehensive biological presentation (gross, microscopic and molecular) and the extensive and updated epidemiological data on each parasite speak of the rigorous scholarship of the contributors and the editors. It should have a special place in all public and private health libraries.

This book makes accessible to medical, public health and other paramedical students and to various health professionals and policy makers important and relevant scientific information on parasites that impact on human

health in the Philippines. It must be a valuable reference for those involved in the eradication of parasitism in communities especially among school children and for all with interest in tropical diseases.

Our teachers have been used to prescribing foreign textbooks in tertiary education and professional courses. This is primarily due to a mindset that we are not capable of making our own. This textbook is proof that Filipino authors can and should provide the information needed by our students, professionals and policy makers. Learning, practice and policy making should, after all, be in the context of what is obtaining in the life and the environment of the learner and user.

UP Manila is particularly proud to be the publisher of this textbook.

MARITA V. T. REYES
Chancellor
University of the Philippines Manila

Foreword to the First Edition

It is a great pleasure and honor to write the foreword of a book which addresses a significant need for information. Definitely, there is a need to make information on medically important parasites more accessible.

The first Philippine Textbook of Medical Parasitology is relevant because it is focused on medical parasites which are found in our local setting. It is therefore an excellent complement to existing books on parasitology which are foreign in orientation.

This book is a welcome addition to locally published learning resources which at the moment are quite meager. We realize the difficulties and travails of editors and authors. I congratulate Dr. Vicente Y. Belizario, Jr. and his team for their commitment and dedication to our countrymen. It is timely that this book is published in 1998, the 100th year of the Republic and the 90th year of the University of the Philippines Manila.

Parasitic infections constitute a major public health problem in the Philippines and many parts of the world. No geographic area is spared from colonization by parasites. The seriousness of the problem is not only confined to the morbidity and mortality that parasites can cause. Its effects are also linked to different aspects of societal life such as decreased productivity and growth, mental retardation, and malnutrition.

All attempts should be made to control parasitic diseases because of their overwhelming ill effects. These have to be a multidisciplinary undertaking requiring, contributions from parasitologists, anthropologists, ecologists, immunologists, clinicians and economists, to name a few.

It is my wish that this book receive the attention it deserves because the knowledge it contains is a powerful means to combat parasitism in our country.

PERLA D. SANTOS-OCAMPO
Chancellor
University of the Philippines Manila

Preface

Parasitic infections remain as a major challenge to public health especially in developing countries like the Philippines. While there have been significant advances in terms of a better understanding of the epidemiology of these infections, improved diagnostic tools and newer approaches to control, in many areas where these infections are encountered, barriers to early diagnosis, treatment, control and prevention remain.

The development of this learning resource, Medical Parasitology in the Philippines, is a response to these continuing challenges. More than providing basic information for students and trainees in medicine, public health, nursing, medical technology, and other allied health professions, this book provides important updates of chapters included in the first two editions of the Philippine Textbook of Medical Parasitology as well as an introduction to important subject areas like neglected tropical and parasitic infections and emporiatrics. This book therefore may be considered as the third edition of the book.

In this edition, the life cycles developed by the United States Centers for Disease Control and Prevention are utilized, and as in the other earlier editions, Bench Aids developed by World

Health Organization are included for reference purposes. For the first time, relevant policies and guidelines from the Department of Health are listed for the guidance of the readers.

The production of this book would not have been possible if not for the major efforts of the members of the Editorial Team as well as the various contributors of the chapters and sections who are themselves experts in their own respective fields. Prof. Winifreda de Leon, with her long experience in parasitic infections, remains the Co-Editor of this book, while Dr. Edsel Maurice Salvaña and Dr. Francis Isidore Totañes served as Associate Editors. Mr. Paul Lester Chua provided vital assistance to push this book writing project forward. The Editorial Team is very grateful to Johnson & Johnson Corporate Contributions Committee that provided a grant for the book writing initiative in a similar way that it provided support for the development of the first two editions of the book.

With the launching of this book, may there be hope that parasitic infections in this beloved country would be better understood, diagnosed, treated, controlled and prevented for a healthier and more productive populace.

VICENTE Y. BELIZARIO, JR.

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We are tremendously indebted to the individual chapter and section contributors. Your expertise and dedication to your profession of teaching and research are the heart and soul of this book. The chapters and sections herein

will lay the foundation for a life of learning in medical parasitology for the next generation of leaders in this field. Working with you has been a great honor and privilege.

Our deepest gratitude to the University of the Philippines Press for providing the technical expertise and know-how to produce an excellent learning resource.

Very special thanks to the Chancellor of the University of the Philippines Manila, Dr. Manuel B. Agulto, for his wholehearted support of this book writing initiative that will provide a valuable reference and guide for students and health professionals in the service of the Filipino people.

We are most grateful to our respective families for their understanding and encouragement in the course of preparing this book. Thank you for allowing us to work more than the usual office hours and beyond the confines of our workplace.

And finally, we give thanks for the enlightenment and guidance from the Almighty, to Whom this work is humbly offered.

VICENTE Y. BELIZARIO, JR.
WINIFREDA U. DE LEON

CHAPTER 1

Introduction to Medical Parasitology

General Considerations

Vicente Y. Belizario, Jr.

Parasitology is the area of biology concerned with the phenomenon of dependence of one living organism on another. *Medical Parasitology* is concerned primarily with parasites of humans and their medical significance, as well as their importance in human communities. *Tropical Medicine* is a branch of medicine that deals with tropical diseases and other special medical problems of tropical regions. A *tropical disease* is an illness, which is indigenous to or endemic in a tropical area but may also occur in sporadic or epidemic proportions in areas that are not tropical. Many tropical diseases are parasitic diseases.

Biological Relationships

Organisms may develop unique relationships due to their habitual and long associations with one another. These relationships are very important to their survival. *Symbiosis* is the living together of unlike organisms. It may also involve protection or other advantages to one or both organisms.

Different forms of symbiosis may be distinguished on the basis of whether or not the association is detrimental to one of the two organisms. *Commensalism* is a symbiotic relationship in which two species live together and one species benefits from the relationship without harming or benefiting the other. For example, *Entamoeba coli* in the intestinal lumen are supplied with nourishment and are

protected from harm, while it does not cause any damage to the tissues of its host. *Mutualism* is a symbiosis in which two organisms mutually benefit from each other like termites and the flagellates in their digestive system, which synthesize cellulase to aid in the breakdown of ingested wood. *Parasitism* is a symbiotic relationship where one organism, the parasite, lives in or on another, depending on the latter for its survival and usually at the expense of the host. One example of a parasite is *Entamoeba histolytica*, which derives nutrition from the human host and causes amebic dysentery.

Parasites

Parasites are often described according to their habitat or mode of development. A parasite living inside the body of a host is known as an *endoparasite*, whereas a parasite living outside the body of a host is an *ectoparasite*. The presence of an endoparasite in a host is called an *infection*, while the presence of an ectoparasite on a host is called an *infestation*. A parasite is considered erratic when it is found in an organ which is not its usual habitat. Most parasites are *obligate parasites* in that they need a host at some stage of their life cycle to complete their development and to propagate their species. Obligate parasites such as tapeworms depend entirely upon their host for existence. A *facultative parasite* may exist in a free-living state or may become parasitic when the need

arises. A parasite, which establishes itself in a host where it does not ordinarily live, is called an *accidental* or *incidental parasite*. A *permanent parasite* remains on or in the body of the host for its entire life, while a *temporary parasite* lives on the host only for a short period of time. A *spurious parasite* is a free-living organism that passes through the digestive tract without infecting the host.

Hosts

Hosts can be classified into various types based on their role in the life cycle of the parasite. A *definitive* or *final host* is one in which the parasite attains sexual maturity. In taeniasis, for example, humans are considered the definitive host. An *intermediate host* harbors the asexual or larval stage of the parasite. Pigs or cattle serve as intermediate hosts of *Taenia* spp., while snails are hosts of *Schistosoma* spp. If there is more than one intermediate host, these can be classified as first and second intermediate hosts.

A *paratenic host* is one in which the parasite does not develop further to later stages. However, the parasite remains alive and is able to infect another susceptible host. For example, *Paragonimus* metacercaria in raw wild boar meat can pass through the intestinal wall of humans and complete its development. In this case, the wild boar serves as a paratenic host transferring the infective stage to humans. Paratenic hosts are important because they widen the parasite distribution and bridge the ecological gap between the definitive and intermediate hosts.

There are also other animals that harbor the parasite other than definitive, intermediate, and paratenic hosts. These are known as *reservoir hosts*. They allow the parasite's life cycle to continue and become additional sources of human infection. Pigs are reservoirs of *Balantidium coli*, field rats of *Paragonimus westermani*, and cats of *Brugia malayi*.

Humans are not always the final host. Humans may be the most important host in the spread of the disease or an *incidental host* of parasites prevalent in other animals.

Vectors

Vectors are responsible for transmitting the parasite from one host to another. A *biologic vector* transmits the parasite only after the latter has completed its development within the host. A biologic vector is therefore an essential part of the parasite's life cycle. When an *Aedes* mosquito sucks blood from a patient with filariasis, the parasite undergoes several stages of development from first stage larva to third stage larva before the latter (infective stage) is transmitted to another susceptible host. A *mechanical* or *phoretic vector*, on the other hand, only transports the parasite. Flies and cockroaches that feed on fecal material may carry enteric organisms and transfer these to food, which could be ingested by humans.

Exposure and Infection

Majority of parasites are pathogens which are harmful and which frequently cause mechanical injury to their hosts. A *carrier* harbors a particular pathogen without manifesting any signs and symptoms. *Exposure* is the process of inoculating an infective agent, while *infection* connotes the establishment of the infective agent in the host.

The *incubation period* is the period between infection and evidence of symptoms. It is sometimes referred to as the *clinical incubation period*. The *pre-patent period*, also known as the *biologic incubation period*, is the period between infection or acquisition of the parasite and evidence or demonstration of infection.

Autoinfection results when an infected individual becomes his own direct source of infection. In enterobiasis, infection may occur through hand-to-mouth transmission. Infective eggs may end up in the hands by scratching the perianal areas where the gravid females lay their eggs. Alternatively, parasites may multiply internally, such as *Capillaria philippinensis*. *Superinfection* or *hyperinfection* happens when the already infected individual is further infected with the same species leading to massive

infection with the parasite. An alteration in the normal life cycle of *Strongyloides* results in a large increase in worm burden, which may lead to severe debilitation or even death due to an increase in the proportion of rhabditiform larvae that transform into filariform larvae while in the gut.

Sources of Infection

There are various sources of parasitic infections. The most common sources are contaminated soil and water. Lack of sanitary toilets and the use of night soil or human excreta as fertilizer allow the eggs to come in contact with the soil and favor the development of *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, and hookworm. Water may be contaminated with cysts of amebae or flagellates, as well as cercariae of *Schistosoma*. Another possible source of infection is food, which may contain the infective stage of the parasite, as exemplified by a number of trematode and cestode infections. Consumption of undercooked or raw freshwater fish can result in several intestinal and liver fluke infections. Raw crabs are considered a delicacy in areas where paragonimiasis is endemic, while raw *Bulla* snails are associated with *Artyfechinostomum malayanum* infection.

Arthropods can also transmit infection. Mosquitoes are vectors of malaria and filarial parasites. *Triatoma* bugs are carriers of *Trypanosoma cruzi* causing Chagas disease. Sand flies (e.g., *Phlebotomus* spp.) are the natural vectors of all types of *Leishmania*. Other animals, whether wild or domesticated, may also harbor parasites. Cats are direct sources of *Toxoplasma* infection, while rats may be infected with *Hymenolepis nana*.

Other sources of infection include another person, his beddings and clothing, as well as the immediate environment he has contaminated, or even one's self. Asymptomatic carriers of *Entamoeba histolytica* working as food handlers in food establishments may be important sources

of infection. Autoinfection where the infected person himself is the source of infection is seen in the life cycles of *Capillaria philippinensis*, *Enterobius vermicularis*, *Hymenolepis nana*, and *Strongyloides stercoralis*.

Modes of Transmission

Since the most common source of parasitic infection is contaminated food and water, the most likely portal of entry is the mouth. Majority of infections with cestodes, trematodes, and intestinal protozoans are foodborne: *Taenia solium*, *Taenia saginata*, and *Diphyllobothrium latum* from eating food harboring the infective larval stages; *Entamoeba histolytica* and *Giardia lamblia* from drinking water contaminated with cysts; and *Clonorchis*, *Opisthorchis*, and *Haplorchis* through ingesting raw or improperly cooked freshwater fish containing infective larvae.

Skin penetration is another route of transmission. Hookworms and *Strongyloides* enter via exposure of skin to soil, while *Schistosoma* species enter skin via water.

Arthropods also serve as vectors and transmit parasites through their bites. Examples are agents of malaria, filariasis, leishmaniasis, trypanosomiasis, and babesiosis.

Another way of acquiring infection is through congenital transmission. *Toxoplasma gondii* trophozoites can cross the placental barrier during pregnancy. In transmammary infection with *Ancylostoma* and *Strongyloides*, the parasites may be transmitted through mother's milk.

Other ways of acquiring the infection include inhalation of airborne eggs of *Enterobius* and sexual intercourse as in the case of *Trichomonas vaginalis*.

Nomenclature

Animal parasites are classified according to the International Code of Zoological Nomenclature. Each phylum is divided into classes, which are further subdivided into orders, families, genera, and species. At times, the

further divisions of suborder, superfamily, and subspecies are employed. Scientific names are latinized; family names are formed by adding *-idae* to the stem of the genus type; generic names consist of a single word written in initial capital letter; the specific name always begins with a small letter. The names of the genera and species are italicized or underlined when written.

Life Cycle

Through adaptation to their hosts and the external environment, parasites have developed life cycles, which may be simple or complicated. Most parasitic organisms attain sexual maturity in their definitive hosts. Some spend their entire lives within the host with one generation after another, while others are exposed to the external environment before being taken up by an appropriate host. The larval stage of the parasite may pass through different stages in an intermediate host before it reaches a final host. As the life cycle becomes more complicated, the lesser the chances are for the individual parasite to survive.

The perpetuation of a species of parasite depends upon its ability to ensure transmission from one host to the next. The parasite must, therefore, adapt to protect itself from the host's defenses and the external environment, and it must overcome the attrition in the species by producing numerous progeny.

Epidemiologic Measures

Epidemiology is the study of patterns, distribution, and occurrence of disease. *Incidence* is the number of new cases of infection appearing in a population in a given period of time. *Prevalence* is the number (usually expressed as percentage) of individuals in a population estimated to be infected with a particular parasite species at a given time. *Cumulative prevalence* is the percentage of individuals in a population infected with at least one parasite. *Intensity of infection* refers to burden of infection which is related to the

number of worms per infected person. This may be measured directly or indirectly and is also referred to as the worm burden. In the case of soil-transmitted helminths, it can be measured directly by counting expelled worms during treatment, or indirectly by counting helminth eggs excreted in feces. The latter is expressed as the number of eggs per gram (epg).

Clinical consequences of infections or diseases that affect an individual's well-being refer to *morbidity*.

Treatment

Deworming is the use of anthelmintic drugs in an individual or a public health program. *Cure rate* refers to the number (usually expressed as a percentage) of previously positive subjects found to be egg negative on examination of a stool or urine sample using a standard procedure at a set time after deworming. *Egg reduction rate* (ERR) is the percentage fall in egg counts after deworming based on examination of a stool or urine sample using a standard procedure at a set time after the treatment.

Selective treatment involves individual-level deworming with selection for treatment based on a diagnosis of infection or an assessment of the intensity of infection, or based on presumptive grounds. This strategy can be used in whole populations, or in defined risk groups. *Targeted treatment* is group-level deworming where the (risk) group to be treated (without prior diagnosis) may be defined by age, sex, or other social characteristics irrespective of infection status. *Universal treatment* is population-level deworming in which the community is treated irrespective of age, sex, infection status, or other social characteristics. *Preventive Chemotherapy* is the regular, systematic, large-scale intervention involving the administration of one or more drugs to selected population groups with the aim of reducing morbidity and transmission of selected helminth infections.

Coverage refers to the proportion of the target population reached by an intervention. It

could be the percentage of school-age children treated during a treatment day.

Efficacy is the effect of a drug against an infective agent in ideal experimental conditions and isolated from any context. *Effectiveness* is a measure of the effect of a drug against an infective agent in a particular host, living in a particular environment with specific ecological, immunological, and epidemiological determinants. Effectiveness is usually measured by means of qualitative and quantitative diagnostic tests which detect eggs or larvae in feces or urine after an optimal time interval, which is variable for each parasite. Cure rate and egg reduction rate are indicators that are commonly used to measure the reduction in prevalence and reduction in intensity of infection, respectively.

Drug resistance is a genetically transmitted loss of susceptibility to a drug in a parasite population that was previously sensitive to the appropriate therapeutic dose.

Prevention and Control

Morbidity control is the avoidance of illness caused by infections. It may be achieved by periodically deworming individuals or groups, known to be at risk of morbidity.

Information-education-communication (IEC) is a health education strategy that aims to encourage people to adapt and maintain healthy life practices.

Environmental management is the planning, organization, performance, and monitoring of activities for the modification and/or manipulation of environmental factors or their interaction with human beings with a view to preventing or minimizing vector or intermediate host propagation and reducing contact between humans and the infective agent.

Environmental sanitation involves interventions to reduce environmental health risks including the safe disposal and hygienic management of human and animal excreta, refuse, and waste water. It also involves the control of vectors, intermediate hosts, and

reservoirs of disease. It also covers the provision of safe drinking water and food safety; housing that is adequate in terms of location, quality of shelter, and indoor living conditions; facilities for personal and domestic hygiene; as well as safe and healthy working conditions.

Sanitation is the provision of access to adequate facilities for the safe disposal of human excreta, usually combined with access to safe drinking water.

Eradication versus Elimination

Disease eradication is defined as a permanent reduction to zero of the worldwide incidence of infection caused by a specific agent, as a result of deliberate efforts. Once this is achieved, continued measures are no longer needed. On the other hand, *disease elimination* is a reduction to zero of the incidence of a specified disease in a defined geographic area as a result of deliberate efforts. Continued intervention or surveillance measures are still required.

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Host-Parasite Relationships

Vicente Y. Belizario, Jr.

The relationship between parasite and host has gradually evolved through the ages. The process has produced changes in the parasite and in its life cycle, consequently affecting the life of its host.

Adaptation causes changes in the molecular biology, biochemistry, immunology, and structure of the parasite. Parasites that are more specialized have shown the greatest changes, most of which are essential for survival.

The most noticeable adaptations are found in the locomotory and digestive organs. Protozoans belonging to the Phylum Apicomplexa have no locomotory organelles, and these organisms are mostly parasitic. Free-living flatworms have cilia on their epidermis, while parasitic cestodes and trematodes do not have any. Cestodes and trematodes obtain nutrients through their tegument, which is provided with microvilli. Flatworms have highly specialized organs of attachment, such as hooks and suckers, which anchor the parasite inside the body of the host and facilitate tissue migration. The size and shape of the parasite are also adapted for maintaining its hold in the host. Adult *Ascaris* worms maintain their position inside the intestinal wall by constant movement. The integument is thickened to resist enzymes and juices in the digestive tract of humans and to protect against dessication and physical injury. In intestinal flukes, the tegument is covered with spines to prevent abrasion. Special coverings of ova, larvae, and cysts protect the parasite during its free-living stage. These coverings also aid in resisting digestive juices once the parasite is ingested by the host.

Reproductive systems of flatworms are highly elaborate and complicated. All tapeworms and flukes, with the exception of *Schistosoma*

spp. are hermaphroditic, that is, they contain a complete set of male and female organs capable of producing thousands of ova. Furthermore, flukes undergo asexual reproduction in the intermediate hosts to increase the number of progeny.

Parasitic existence may also result in profound biochemical adaptations. Such changes include loss of certain metabolic pathways common to free-living organisms. This process is called streamlining, that is the inability of the parasite to synthesize certain cellular components and the need of the parasite to obtain these from a host. Streamlining is exemplified by hemoflagellates and other helminth parasites. These changes in metabolic pathways may become the target of future chemotherapeutic strategies.

Some parasites have developed specialized mechanisms needed for entry into the body or tissues. The trophozoites of *Entamoeba histolytica* secrete cysteine proteinases, which allow the parasite to penetrate the mucosa and adhere to the underlying layer and surrounding tissues. No such enzyme has been found in the commensal *Entamoeba coli*. The cercariae of *Schistosoma* contain penetration glands, which produce an enzyme capable of digesting the skin allowing entry into the body of the host. All cestode embryos have six hooklets, which aid them in tissue penetration before developing into encysted larvae.

Effects of the Parasite on the Host

Some organisms may live inside the body of the host without causing any damage, but in most instances, they have the ability to inflict damage to their host. There are several mechanisms by which parasites cause injury to the host. The most common mechanism

is by interference with the vital processes of the host through parasitic enzymes. Secretory and excretory products elaborated by many parasites allow them to metabolize nutrients obtained from the host and store these for energy production. This is best exemplified by *Entamoeba histolytica* trophozoites that secrete cysteine proteinases, which do not only digest cellular materials but also degrade epithelial basement membrane facilitating tissue invasion.

Another mechanism is through invasion and destruction of host tissue. One example is *Plasmodium*, which invades red blood cells. After multiplication, the host's red blood cells rupture resulting in the release of merozoites. In *Schistosoma japonicum* infection, cumulative deposition of eggs in the liver stimulates an immune response mechanism resulting in granuloma formation and then fibrosis which leads to portal hypertension and massive hemorrhage in the venules. Hookworms have cutting plates, which can attach to the intestinal mucosa and destroy the villi. Large numbers of worms such as *Ascaris* form tangled masses that can lead to intestinal obstruction. An *Ascaris* worm in the intestine may invade other organs like the appendix and bile ducts and may cause a surgical emergency.

Parasites can also deprive the host of essential nutrients and substances. Heavy hookworm infection causes massive intestinal bleeding which results in chronic blood loss and iron deficiency anemia. *Diphyllobothrium latum* competes with its host for the available supply of Vitamin B₁₂, thus resulting in megaloblastic anemia.

Effects of the Host on the Parasite

There are several factors which determine the outcome of an infection. The genetic make-up of the host may influence the interaction between host and parasite. In falciparum malaria, possession of sickle-cell trait confers some protection, while the presence of Duffy

blood factor increases the susceptibility of an individual to *Plasmodium vivax* infection.

Another important aspect is the nutritional status of the host. A diet rich in protein is not suitable for the development of intestinal protozoans, while a low-protein diet favors the appearance of symptoms of amebiasis and complications of the disease. A high carbohydrate diet favors the development of some tapeworms.

Immune processes play an important role in host-parasite relationships. Absolute immunity to reinfection occurs rarely following protozoan infections, and probably never happens with helminth infections in humans. Acquired immunity may be very important in modifying the severity of disease in endemic areas.

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Immunology of Parasitic Infections

Edsel Maurice T. Salvaña, Winifreda U. de Leon, Katerina T. Leyritana

The function of the immune system is to protect the body from invasion by potential pathogens. It is a tightly-controlled balancing act, in the sense that dysfunction of the immune system can lead to either a permissive environment for infection on one hand, or to unchecked activation which can harm the organism on the other. Immunity to parasites, especially eukaryotes such as helminths and protozoans, is complicated by the fact that, unlike bacterial pathogens, eukaryotic organisms are similar in make-up and physiology. Moreover, parasites have evolved strategies to evade the immune system over millions of years, and some are so successful that these organisms not only survive but thrive in the bloodstream (e.g., *Schistosoma* sp.) where they are subjected to constant and intimate exposure to the body's immune system.

Parasitic infections in humans and animals occur when the parasite successfully establishes itself in the host and is not eliminated by many host defense systems and is able to continue its life cycle. However, not all interactions between the host and parasite relationship result in injury and pathology. It can result in the following outcomes:

- Parasite fails to become established in the host.
- Parasite becomes established and the host eliminates the infection.
- Parasite becomes established, and the host begins to overcome the infection but is not totally successful.
- Parasite becomes established and the host, in trying to eliminate the organism, becomes damaged itself.
- Parasite becomes established and kills the host.

The ability of the parasites to cause infections has evolved through the process of natural selection, since only a proportion of parasites are able to accomplish this. In the same way, the host's ability to defend itself against a parasite's invasion is also selected for. Some life cycles are so complicated that the parasite has adapted means to survive immune assault in not just one but a variety of hosts, including the definitive host, intermediate hosts, and reservoir hosts.

The host-parasite relationship remains dynamic, and while some parasites become specific to some hosts over time, accidental infection of erstwhile non-susceptible hosts may eventually lead to establishment of a new reservoir, intermediate, or definitive host which in time may even become the dominant host for that organism. This is exemplified by zoonoses such as infections with *Trypanosoma* sp., and the newly discovered human malaria parasite *Plasmodium knowlesi*.

Host-Parasite Interactions

Natural physical barriers to the entry of the parasite into the body constitute the first line of defense against pathogens. The skin provides effective surface protection against invasion from parasites that initiate infection through skin penetration. Adaptive mechanisms of some helminths allow them to overcome these defenses. The filariform larvae of hookworms and *Strongyloides* can synthesize a protein that aids in the entry through the skin. *Schistosoma* spp. cercariae are capable of skin penetration because of the presence of glands in the anterior part of the parasite that secrete lytic enzymes.

The mucous membranes lining the respiratory, gastrointestinal, and genitourinary tracts provide external barriers to parasite entry

as well. Tight junctions between epithelial cells serve to prevent passage of all but the smallest molecules. The low pH of vaginal secretions and gastric juices present a hostile environment to many microorganisms. For instance, the trophozoites of *Trichomonas vaginalis* are unable to survive the acidic environment of the vagina, and once intestinal secretions envelope *Giardia lamblia*, its motility is greatly diminished reducing injury to the host. To evade this type of host defense, the infective stages of helminths that are ingested, like embryonated eggs of *Ascaris*, *Trichuris*, and *Taenia* spp. are protected from the acidic environment by thick egg shells. The cystic wall of intestinal protozoa like the *Entamoeba* and *Giardia* are also resistant to acidic pH.

Chemical components of body fluids play a major role in the protection of the host. The lipase content of breast milk, for example, has been found to be toxic to *Giardia lamblia* *in vitro*. Lysozyme found in tears and saliva is able to destroy microorganisms, along with secreted IgA immunoglobulins in these fluids.

Physiologic functions of the body also inhibit parasite invasion. Peristalsis, motion of cilia, and human reflexes all serve to expel parasites. Coughing enables expectoration of aberrantly situated adult *Ascaris lumbricoides* and eggs of *Paragonimus westermani*, and the flushing action of urine decreases the numbers of *Trichomonas vaginalis*.

In the event that the parasite is able to overcome physical barriers, a second host defense comes into play. The penetration of the body's barriers results in a series of events that facilitate sensing of the invading parasite via pathogen-associated molecular patterns, or through pattern recognition responses which enable the body to mount an immune response that acts towards eliminating or limiting the infection.

Host-Immune Response

The host possesses both innate and acquired immune defenses. Both kinds of

defenses rely on humoral and cell-mediated mechanisms of action.

The innate response happens when the body detects and eliminates pathogens through non-specific mechanisms that use mechanical, chemical, and cytokine-mediated methods to destroy or disrupt invading organisms with little or no delay from the time of invasion. One method is through phagocytosis by macrophages and dendritic cells with subsequent pathogen elimination through oxidative killing and use of toxic peptides. Some intracellular pathogens are able to invade and multiply inside macrophages, like *Leishmania* spp., *Toxoplasma gondii*, and *Trypanosoma cruzi*, in which case cell-mediated immune mechanisms (whether non-specific such as natural killer cells, or acquire cell-mediated immunity through T-lymphocytes) are required to identify and destroy them.

Toll-like receptors (TLRs) recognize specific molecules that are non-native to the body and so represent some of the earliest recognition mechanisms for pathogens. To date, ten TLRs have been identified and each is activated by a bacterial components [e.g., LPS (TLR4), diacylated lipoprotein (TLR2 and 6) and triacylated lipoprotein (TLR 1 and 2), flagellin (TLR5)], viral RNA (TLR3), and other unfamiliar components. Binding of a specific ligand to a TLR causes a cascade of reactions down a common signaling pathway which produces cytokines such as interferon gamma and interleukin-1. These cytokines activate natural killer cells and macrophages, stimulation of which leads to further production of inflammatory cytokines, and co-stimulatory molecules. TLRs are therefore largely responsible for triggering the initial inflammatory response. They function as pyrogens and synthesize inflammatory response proteins, which then increase the number and function of phagocytic cells.

The host, once infected, is exposed to the parasite antigens, which in turn can stimulate the host to mount an acquired specific response against the antigen. The expression of acquired

immunity is the result of a complex series of immunoregulatory events: activation, induction through proliferation, differentiation, and effector function. The effector function may be at the end point of a response or it might serve a regulatory function that modulates other functions.

The parasitic antigens may originate from the surface, from secretions and excretions, and from somatic tissues of the parasite. Following initial contact with antigen (immunologic priming), subsequent antigen exposure leads to more rapid and vigorous immune responses, leading to immunologic memory. The response of acquired immunity is either antibody-dependent or cell-mediated.

Most of the time, immunity is directed against the antigen that induced the response. Cross-reactivity does occur. The antigen may be present in just one developmental stage or in just one species of the parasite. There are antigens, however, that have been detected in all of the stages of parasite development or in all members of a genus. It is therefore important to remember that an immune response does not always equate with protection, and that conversely, immunity to one pathogen may confer immunity to another closely related species.

Acquired Immune Response

The immune response to parasitic infections is under well-defined genetic control and has a strong influence over the outcome of infection in terms of resistance, susceptibility, and pathology. The *major histocompatibility complex (MHC)* gene products help regulate T-lymphocyte activities. *Human leukocyte antigen (HLA)* is also a factor.

The specific immune response to the parasite begins when parasitic antigens are processed and presented to the CD4 T-helper lymphocytes, which either belong to the Th1 or Th2 subset. These subsets of T-helper cells are responsible for producing different lymphokines.

Th1 lymphocytes produce gamma interferon and interleukin-2 which activate cytotoxic lymphocytes (with CD8 surface molecules) and macrophages. This brings about the cell-mediated immune response.

Cell-mediated immunity has been observed in many parasitic infections. Parasite-specific antigens induce clonal expansion of parasite-specific T-lymphocytes. They may act by direct cytotoxicity on the parasite or indirectly by acting on natural killer cells or the antibody producing B-lymphocytes. Migrating larvae of *Toxocara canis* are killed through cell-mediated activity.

Th2 lymphocytes produce interleukins 4, 5, and 6 that enhance the proliferation and differentiation of B-lymphocytes into plasma cells, which are responsible for immunoglobulin production. The antibodies that are produced bind with specific parasite antigens and can activate complement and include the following classes: IgE, IgG, IgM, and IgA.

In helminthic infections, the most common responses include eosinophilia and elevated serum IgE. With lumen-dwelling *Ascaris lumbricoides* and *Trichuris trichiura*, however, the immune response is not as intense compared with lymphatic dwelling *Wuchereria bancrofti* and *Brugia malayi* since contact with both recognition and effector elements of the immune system is less intimate. Immunologic response is also marked in visceral larval infections with *Parastrongylus cantonensis* and *Toxocara canis* which are less likely to have immune-evading mechanisms since they are not specifically adapted to the human host.

IgE antibodies that are bound to the mucosal mast cells, eosinophils, and goblet cells can mediate the eventual expulsion of adult gastrointestinal helminths. IgE has also been identified on inflammatory cells involved in the cytotoxic action on some parasites like *Schistosoma* spp. referred to as antibody dependent cell-mediated cytotoxicity (ADCC). There are a variety of activating molecules expressed by the eosinophils that mediate

ADCC. Among these are eosinophil activating factor (EAF), interleukin-5, and granulocyte-monocyte colony stimulating factor (GM-CSF). Destruction of microfilariae among patients with tropical pulmonary eosinophilia has been attributed to ADCC mediated by IgE and eosinophils. Cells like neutrophils and platelets have been found to participate in ADCC as well.

With homocytotropic IgG1, IgE can act on mast cells and basophils, which can lead to degranulation and eventual release of pharmacologically active substances. Unregulated activation can result in an anaphylactic Type 1 hypersensitivity reaction as seen during the rupture of *Echinococcus granulosus* hydatid cysts. The same immediate hypersensitivity reaction has been observed at the site of the bite of several arthropods like mites and ticks.

The combined activity of IgG and IgM can prevent penetration of erythrocytes by *Plasmodium* spp. and *Babesia* spp., but are generally ineffective against gastrointestinal helminths. In the presence of complement activity, these antibodies can mediate lysis of trypomastigotes of *Trypanosoma cruzi* and, even in the absence of the complement, are involved in the rapid phagocytosis of the same parasites.

Secretory IgA in the intestines protect against metacystode and gastrointestinal infections. IgM with secretory IgA mediate ADCC in *Giardia lamblia* infection. Among immunocompetent individuals, *Cryptosporidium* infection is self-limited due to the combined action of IgA and IgG with cell-mediated immunity, which helps cleave the parasite from the enterocytes.

In many infections, be it microbial or parasitic, the host can activate its non-specific, specific, humoral, and cell-mediated defenses all at the same time.

Parasite Evasion Mechanisms

Parasites have several characteristics that make it difficult for the host to detect and eliminate them: parasite size, complicated

parasite life cycles, location within body sites that are relatively protected from the immune response, and antigenic complexity.

In addition, natural selection and adaptation have resulted in deployment by the parasite of various mechanisms to avoid the destructive effect of the host response. These major mechanisms include induction of immune suppression, antigenic variation, host mimicry, and sequestration among others.

A. Resistance to Immune Response

Protozoa and helminthic parasites that enter the blood stream or tissue are often able to survive and replicate because they are resistant to the host innate immune response. Parasites in humans are usually resistant to complement. Macrophages can phagocytose protozoa, but the cuticle and integument of helminthic parasites make them resistant to the cytotoxic effects of both neutrophils and macrophages. This may be due to the loss of surface molecules that bind complement or acquisition of host regulatory proteins such as decay accelerating factor. Trypanolytic factors such as apolipoprotein L-1 (APOL1) destroy non-human trypanosomes except *Trypanosoma brucei* which has evolved resistance through expression of serum resistance-associated protein. A frameshift mutation in the APOL1 gene enables a non-human trypanosome (*T. evansi*) to infect a human, and addition of recombinant APOL1 restored trypanolytic activity.

B. Immune Suppression

There are parasites that can reduce the immune function of macrophages that result in lower capacity of phagocytosis and defective processing of antigen, as in the case of *Plasmodium* spp. infection. In *Trypanosoma brucei* infection, the trypomastigotes can produce large amounts of surface glycoproteins. This affects the processing of the proteins due to antigenic competition and at the

same time impairs the B- and T-lymphocyte activities resulting in diminished production of lymphokines and immunoglobulins.

Entamoeba histolytica suppresses macrophage respiratory burst and consequent nitric oxide production, produces a suppressor factor that can inhibit movement of monocytes to the site of invasion (monocyte locomotion inhibitory factor), and inhibits complement assembly. In *Fasciola* infection, there is down regulation of Th1 lymphocytes. In filarial infections with *Wuchereria bancrofti* and *Brugia malayi*, there is polyclonal hypergammaglobulinemia where antibodies lack specificity against these parasites. This has also been observed in *Plasmodium* spp. infection.

Blocking antibodies produced by several parasites like *Wuchereria bancrofti* can also dampen the effect of immune responses. In *Necator americanus* infection, the immune response is directed against the deeper layers of its cuticle but the immune response is diverted to the rapidly changing surface of its integument.

Immune complexes produced in cysticercus cellulosae infection suppress inflammatory response through inhibition of complement activity. Infection with *Plasmodium* spp. and *Trypanosoma cruzi* can also lead to immunosuppression through the production of immune complexes. In *Schistosoma* spp. infection, complement cannot participate in the destruction of the parasite; it has been found that the complement is consumed by the soluble antigens of the *Schistosoma* spp.

C. Antigenic Variation

In *Trypanosoma brucei* infection, the initial host response against the surface glycoproteins of the trypomastigotes is very effective. But in the subsequent releases of trypomastigotes, the immune response is no longer effective since the parasites have changed the antigenic profile of their surface coat through variant surface glycoproteins (VSG). Surface protein variation has also been observed in *Giardia lamblia*.

Malarial parasites, especially *Plasmodium falciparum*, exhibit antigenic diversity. The mechanism is through repeat variation of the encoded polypeptides, which contain tandem sequences of amino acids, as observed in merozoite surface antigen (MSA) and ring-infected erythrocyte surface antigen (RESA). These repeat sequences are antigenic epitopes, which stimulate antibody production. With variation, therefore, antibodies fail to recognize the antigen.

D. Host Mimicry

The larval stage of *Echinococcus granulosus* in the hydatid cyst has been found to carry P blood group antigen, and the tegument of *Schistosoma* spp. adult can acquire antigenic molecules from the host. Antibodies produced against the parasite then fail to recognize non-self from self-antigens.

E. Intracellular Sequestration

Amastigotes of *Trypanosoma cruzi* and *Leishmania* spp. proliferate in macrophages in various organs. *Toxoplasma gondii* multiply inside macrophages as well as in other nucleated cells. Once intracellular, they are able to evade the host immune response.

The late intracellular stages of *Plasmodium falciparum* are sequestered from the circulation in deep vasculature beds. This is mediated by the presence of knobs on infected erythrocytes that enable them to attach to endothelial cells of capillaries. This sequestration process excludes the parasitized red blood cells from splenic filtration and the action of antibodies.

Adverse Effects of the Immune Response in the Host

Under normal circumstances, orderly progression of host defenses through the different phases results in a well-controlled immune and inflammatory response that protects the host from the offending antigen. However, dysfunction of any of the host defense systems can result in damage to host tissue and

produce clinical disease. The normal immune response itself might contribute substantially to tissue damage as one of four types of reactions: Type 1 (Immediate type hypersensitivity), Type 2 (Immune complex formation), Type 3 (Cytotoxic reactions of antibody), and Type 4 (Delayed-type hypersensitivity).

In acute infection with *Trypanosoma cruzi*, the intense immune response to the parasite is accompanied by massive damage not only to the infected cells but also to the surrounding cells including nerve cells and myocytes. It is believed that this is partially responsible for heart failure and meningoencephalitis. Moreover, it has been postulated that antibodies to *T. cruzi* may activate adrenergic and muscarinic receptors because of similarities between these and parasite antigens, leading to autonomic dysfunction and predisposition to arrhythmias. In *Wuchereria bancrofti*, there is an overproduction of IgM (polyclonal hypergamma-globulinemia) due to the functional T-suppressor cell (T8) defect, which explains the formation of a large amount of immune complexes in Tropical Pulmonary Eosinophilia (TPE).

In recurrent *Plasmodium* spp. infection, immune complexes are associated with a condition called hyperactive malarious splenomegaly (HMS). There is a disturbance in the ability of T-lymphocytes to control the humoral response resulting in polyclonal IgM antibodies. Patients suffer from persistent splenomegaly and anemia. In patients with *Plasmodium malariae* infection, these immune complexes may be deposited in the basement membrane of the glomeruli leading to kidney failure and nephrotic syndrome. This phenomenon may also occur in schistosomiasis.

While the sequestration of late intraerythrocytic *Plasmodium falciparum* from the circulation and their attachment to endothelial cells is protective to the parasite, this is also believed to be the main cause of manifestations of cerebral malaria.

The main clinical manifestations of *Schistosoma* spp. infection are related to the host immune response to eggs that are trapped in various organs of the host. This usually results in hepatosplenomegaly, fibrosis, portal hypertension, and esophageal varices. High levels of *Schistosoma* spp. circulating antigen in immune complexes can produce a condition very similar to serum sickness. T-cell mediated delayed-type of hypersensitivity lymphocytes, when stimulated such as in *Schistosoma* spp. infection, can produce attractants and activators of other cells that form destructive granulomas around *Schistosoma* spp. eggs. In *Leishmania* spp. infections, more macrophages are damaged, be it the cutaneous, mucocutaneous, or visceral type of infection.

Practical Applications

Understanding the host immune response to parasitic invasion is useful in immunodiagnosis, and predicting the resulting pathology. Current concepts on immunoregulation and immunomodulation are products of intense and meticulous studies on these immune mechanisms. These insights may hold the key for potential control through vaccination and development of novel anti-parasitic drugs.

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Groups of Parasites with Medical and Public Health Importance

Winifreda U. de Leon, Vicente Y. Belizario, Jr.

All parasites can be classified according to the Linnaean hierarchical scheme in order of decreasing generality. It starts with Kingdom, Subkingdom, Phylum, Class, Order, Family, Genus, and finally, Species. This hierarchical classification is mainly based on morphological characterization found in the different stages of parasite development.

Currently, however, there are powerful tools based on molecular studies which may provide elucidation of the taxonomic relationship of parasites at the subcellular level. Molecular techniques such as DNA extraction and sequencing, proteome analysis, RNA interference, and polymerase chain reaction are being used to show structural differences among parasites. These are especially useful in the identification of cryptic protozoan parasites and their sibling species. Currently, there is a call for parasitologists to integrate molecular and morphological approaches in the identification of parasites. This chapter will not elucidate on these molecular advances, but the reader is enjoined to explore the included references at the end of the chapter for further details.

Protozoa

Parasitic infections are either due to the unicellular protozoan or the multi-cellular metazoan. Generally, protozoan parasites are provided with a nucleus or nuclei, cytoplasm, an outer limiting membrane, and cellular elaborations called organelles. Among these are locomotory apparatus, which include cilia, flagella, and pseudopodia. There is increasing knowledge about the presence of an apical complex found to aid the organism in the penetration of target cells.

Many of these protozoa require a wet environment for feeding, locomotion, osmoregulation, and reproduction. They form

infective stages called cysts, which are relatively resistant to environmental changes compared to the vegetative stages, called trophozoites. The parasitic species are capable of multiplying within the host and may be transmitted through a biological vector within which they can also multiply (Table 1.1).

All protozoa fall under Kingdom Protista, which is a diverse group of eukaryotic microorganisms. They have been divided into several phyla, but the major organisms causing disease in man belong to Phylum

Table 1.1. Classification of protozoan parasites

Sarcomastigophora Sarcodina	<i>Acanthamoeba castellanii</i> <i>Endolimax nana</i> <i>Entamoeba coli</i> <i>Entamoeba dispar</i> <i>Entamoeba gingivalis</i> <i>Entamoeba histolytica</i> <i>Iodamoeba butschlii</i> <i>Naegleria fowleri</i>
Mastigophora Atrial flagellates	<i>Chilomastix mesnili</i> <i>Dientamoeba fragilis</i> <i>Giardia lamblia</i> <i>Trichomonas hominis</i> <i>Trichomonas tenax</i> <i>Trichomonas vaginalis</i>
Hemoflagellates	<i>Leishmania braziliensis</i> <i>Leishmania donovani</i> <i>Leishmania tropica</i> <i>Trypanosoma brucei complex</i> <i>Trypanosoma cruzi</i>
Ciliophora	<i>Balantidium coli</i>
Apicomplexa	<i>Babesia</i> spp. <i>Cryptosporidium hominis</i> <i>Cyclospora cayetanensis</i> <i>Cystoisospora belli</i> <i>Plasmodium</i> spp. <i>Toxoplasma gondii</i>
Microspora	<i>Enterocytozoon bieneusi</i> <i>Encephalitozoon</i> spp. <i>Vittaforma cornea</i> <i>Trachipleistophora hominis</i> <i>Pleistophora</i> spp. <i>Anncaliia vesicularum</i> <i>Microsporidium</i> spp.

Sarcomastigophora, Phylum Ciliophora, Phylum Apicomplexa, and Phylum Microspora.

Under Phylum Sarcomastigophora are two subphyla, namely, Subphylum Mastigophora, whose organelles of locomotion are whip-like structures arising from the ectoplasm called flagella, and Subphylum Sarcodina, whose organelles of locomotion are hyaline foot-like extrusions from the ectoplasm called pseudopodia. Subphylum Mastigophora includes the atrial flagellates and hemoflagellates, namely, *Giardia*, *Chilomastix*, *Trichomonas*, *Dientamoeba*, *Trypanosoma*, and *Leishmania*. Subphylum Sarcodina includes the amebae, namely, *Entamoeba*, *Endolimax*, *Iodamoeba*, *Acanthamoeba*, and *Naegleria*. Phylum Ciliophora, whose species have organelles of locomotion that are hair-like projections from the ectoplasm called cilia, which includes only one parasite of medical and public health interest, *Balanitidium coli*.

Members of Phylum Apicomplexa have an apical complex at the anterior end which consists of polar rings, subpellicular tubules, conoid processes, rhoptries, and micronemes. These structures are involved in the penetration and invasion of target cells. All members are parasitic. Very important groups of parasites fall under Class Sporozoa, namely, *Plasmodia*, *Babesia*, *Toxoplasma*, *Cystoisospora*, *Cryptosporidium*, and *Cyclospora*. These organisms have been reported practically from all organ systems of both humans and animals, specifically in the gastrointestinal tract, genitourinary tract, central nervous system, respiratory tract, reticuloendothelial system, blood and blood cells, eyes, skin, and even the oral cavity.

Phylum Microspora, which includes *Enterocytozoon* and *Encephalitozoon*, consists of spore-forming parasites of both vertebrates and invertebrates. Though the phylum contains more than 100 genera, the members are similar, in that they possess a unique extrusion apparatus which enables them to insert infective material into the host cell. The apparatus includes a highly coiled polar filament, which, due to

varying stimuli from the gastrointestinal tract, extrudes, forming a polar tube that, in turn, penetrates the host cell. These parasites have received more attention recently due to the increasing number of opportunistic infections associated with immunocompromised states, particularly AIDS.

Nematodes

Metazoan parasites are either helminths or arthropods which fall under the Kingdom Animalia (Table 1.2). Helminths causing

Table 1.2. Classification of metazoan parasites

Nematoda	Intestinal	<i>Ascaris lumbricoides</i> <i>Capillaria philippinensis</i> <i>Enterobius vermicularis</i> Hookworm <i>Strongyloides stercoralis</i> <i>Trichuris trichiura</i>
	Extraintestinal	Lymphatic filarial <i>Parastrongylus cantonensis</i> <i>Trichinella spiralis</i>
Cestoidea	Cyclophyllidea	<i>Dipylidium caninum</i> <i>Echinococcus</i> spp. <i>Hymenolepis diminuta</i> <i>Hymenolepis nana</i> <i>Raillietina garrisoni</i> <i>Taenia saginata</i> <i>Taenia solium</i>
	Pseudophyllidea	<i>Diphyllobothrium latum</i> <i>Spirometra</i> sp.
Trematoda		<i>Artyfechinostomum malayanum</i> <i>Clonorchis sinensis</i> <i>Echinostoma ilocanum</i> <i>Fasciola hepatica</i> <i>Fasciolopsis buski</i> <i>Heterophyids</i> <i>Opisthorchis felineus</i> <i>Opisthorchis viverrini</i> <i>Paragonimus westermani</i> <i>Schistosoma haematobium</i> <i>Schistosoma japonicum</i> <i>Schistosoma mansoni</i>
Arthropoda		Mites
Arachnida		Scorpions Spiders Ticks
Chilopoda		Centipedes
Crustacea		Copepods, Crabs
Diplopoda		Millipedes
Insecta		Flies, Flea, Beetle, Bees, Lice, Wasp, Bugs, Mosquitoes
Pentastomida		Tongue Worms

infections in man belong to three groups, namely, annelids, nematodes, and flatworms. Under the annelids, only the leeches are considered to be of medical importance.

The nematodes are also known as roundworms because they are elongated and cylindrical in shape, with bilateral symmetry. Generally, they have a complete digestive tract and a muscular pharynx that is characteristically triradiate. They are provided with separate sexes, although some may be parthenogenetic. There are sensory organs in the anterior and posterior ends of the worm called amphids and phasmids, respectively. The latter are very useful in the grouping of the nematodes. Those roundworms with phasmids are described as phasmid nematodes, while those without them are described as aphasmid worms. Among the nematodes of medical and public health importance, only three are aphasmid worms (Adenophorea). These are *Trichuris*, *Trichinella*, and *Capillaria*. The rest of the nematodes are, therefore, phasmid nematodes (Secernentia).

The phasmid worms belong to several orders in the scientific taxonomic classification of the worms. *Ascaris* belongs to Ascaridida, *Parastrongylus* and the hookworms to Strongylida, *Strongyloides* to Rhabditida, *Enterobius* to Oxyurida, and the filarial worms to Spirurida. A more extensive discussion of the taxonomic groupings of these worms can be found in other references.

These nematodes can be grouped on the basis of the habitat of the adult worms. Most of these nematodes are found in the small and large intestines, while some are found outside the intestines.

Those typically found in the small intestines are *Ascaris*, hookworms, *Strongyloides*, and *Capillaria*, while those usually located in the colon are *Trichuris* and *Enterobius*. Extraintestinal nematodes like *Wuchereria* and *Brugia* have been recovered from the lymph nodes and lymph vessels, whereas *Parastrongylus* has been reported from the eyes and meninges.

Larvae of *Trichinella* are encysted in the host muscles.

There are various ways by which humans acquire these helminths. Ingestion of embryonated eggs is the mode of infection of *Ascaris*, *Trichuris*, and *Enterobius*. Skin penetration by filariform larvae is the mode of infection of hookworms and *Strongyloides*, while the bite of mosquito vectors is the mode of transmission of *Wuchereria* and *Brugia*. Ingestion of infective larvae is the mode of infection for *Capillaria* from fish, *Trichinella* from pork, and *Parastrongylus* from snails. Autoinfection occurs in *Capillaria*, *Strongyloides*, and *Enterobius*. Transmission through inhalation of embryonated eggs is possible for *Enterobius* and *Ascaris*.

Cestodes

The two other groups of worms are tapeworms or cestodes, and flukes or trematodes. These belong to Platyhelminthes or the flatworms. Members of Platyhelminthes, in general, are dorso-ventrally flattened with bilateral symmetry. The cestodes are segmented, with a ribbon-like appearance, while the trematodes are leaf-like and unsegmented. Cestodes do not have a digestive tract, while trematodes have an incomplete one. Both cestodes and trematodes do not have a circulatory system.

Adult tapeworms are hermaphroditic. They are found in the intestines of the definitive host, and the larval stage is encysted in the tissues of the intermediate host. They have an anterior structure called the scolex, which is the main organ of attachment of the worm to the definitive host. After the scolex is the neck, which is then followed by the strobila. The neck is considered the region of growth, because segmentation or strobilization originates from it. Segments or proglottids that are nearest to the neck are the most immature, followed by increasingly mature segments, and the most distal are gravid segments.

The cestodes are grouped together into different orders, just like the nematodes. However, there are only two orders of tapeworms with medical and public health significance, namely, Order Pseudophyllidea and Order Cyclophyllidea. These two orders differ in terms of the morphology of the scolex, segments, and eggs, as well as in the number of intermediate hosts and the type of encysted larvae that develop in the intermediate hosts.

Pseudophyllidean tapeworms have a spatulate scolex with sucking grooves, called bothria, while the Cyclophyllidean scolex is globular with four muscular suckers. Segments of both orders have genital pores but Pseudophyllidean segments, in addition, have a uterine pore which allows release of eggs from the gravid uterus. Since Cyclophyllidean segments do not have the uterine pore, they undergo the process of apolysis whereby gravid segments are detached from the main body of the worm and eggs are eventually released. For diagnostic purposes, in Cyclophyllidean infections, both eggs and segments are recovered from the patients, while in Pseudophyllidean infections, segments may not be found.

Non-operculated Cyclophyllidean eggs are passed out readily, containing the hexacanth embryo. On the other hand, Pseudophyllidean eggs, which are operculated and immature, require aquatic development of the embryo, called the coracidium.

Pseudophyllidean worms generally require two intermediate hosts in their life cycle. In the first intermediate host, eggs encyst as proceroid larvae, then into plerocercoid larvae in the second intermediate host. This group of tapeworms is best represented by *Diphyllobothrium*, which utilizes humans as definitive hosts, and *Spirometra*, which employs humans as an intermediate host.

Cyclophyllidean worms require only one intermediate host, but different species of Cyclophyllideans produce different types of encysted larvae in the intermediate hosts. The

various species of *Taenia* produce the cysticercus type, while *Hymenolepis*, *Dipylidium*, and *Raillietina* produce the cysticercoid type. A third type called the hydatid is produced by *Echinococcus* spp.

Infection with adult tapeworms is generally acquired through the consumption of infected intermediate hosts. There are cases, however, where humans are infected with the larval stage of *Taenia solium*, called cysticercosis, and of *Echinococcus* spp., called hydatid cyst.

Trematodes

The other group of flatworms is composed of the flukes or trematodes. Adult trematodes are equipped with an oral sucker, and a ventral sucker called an acetabulum. A third sucker called a genital sucker or gonotyl is observed only among the heterophyids. They are all hermaphroditic. All trematodes require two intermediate hosts in their life cycle. All trematodes have operculated eggs, and the infective stage for all these trematodes is the encysted larva, the metacercaria, that develops in the second intermediate host. These characteristics are observed in all medically important trematodes, with the exception of the schistosomes in which the infective stage is the cercaria. While the first intermediate host is always a snail, the second intermediate host may be a fish, crustacean, another snail, or fresh water plants.

Trematodes are generally grouped together based on their habitat. Adult schistosomes are found in the mesenteric veins; hence they are called blood flukes. Adult *Paragonimus* worms are found in the lung parenchyma. There is a group of flukes that inhabits the liver and bile passages. This group includes *Fasciola*, *Clonorchis*, and *Opisthorchis*. Another group composed of *Fasciolopsis*, *Echinostoma*, and heterophyids inhabits the intestines.

Mature eggs contain an embryo called the miracidium. Eggs passed out by an infected host may be mature, as in the case of *Schistosoma*,

Clonorchis, *Opisthorchis*, and heterophyids; while immature eggs are associated with *Paragonimus*, *Fasciola*, *Fasciolopsis*, and *Echinostoma*. The miracidium of immature eggs develops in an aquatic environment.

Arthropods

Phylum Arthropoda is composed of bilaterally symmetrical organisms with segmented and jointed appendages. The body is covered with a chitinous exoskeleton. This group includes insects, mites, ticks, spiders, scorpions, centipedes, millipedes, and crustaceans. Pentastomids or pentastomes may be included under this group because they have the attributes of both arthropods and annelids.

Arthropods affect human health in various ways, like envenomization through bites of spiders, flies, bugs, mites, and ticks. Introduction of venom can also occur with stings of scorpions, ants, wasps, and bees. Exposure to arthropod allergens has recently been recognized as a health hazard. There are arthropods that feed on human blood, like biting flies and mosquitoes that enable them to become biological vectors to some disease agents like *Plasmodium*, filaria, trypanosomes, *Babesia*, and *Leishmania*. On the other hand, flies and cockroaches, which inhabit unsanitary environments, can be mechanical vectors of microbes and parasites.

Some arthropods, such as fleas and lice, can cause dermatologic manifestations due to prolonged contact with the human hosts. Fly larvae can cause infestation and invasion of human tissues, a condition known as myiasis.

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CHAPTER 2

Protozoan Infections

Intestinal Amebae

Pilarita T. Rivera, Windell L. Rivera, Juan Antonio A. Solon

Seven species of amebae occur in humans. These include the pathogenic *Entamoeba histolytica*, and the commensals *E. dispar*, *E. moshkovskii*, *E. hartmanni*, *E. coli*, *Endolimax nana*, and *Iodamoeba butschlii*. *Entamoeba polecki* is an intestinal ameba of pigs and monkeys that has been occasionally detected in humans, and is a probable cause of diarrhea. They are mainly differentiated on the basis of structure and size. Trophozoites divide by binary fission. Most cyst-forming amebae go through nuclear division, and then divide again after excystation in a new host.

Entamoeba histolytica

Entamoeba histolytica is currently classified within the subphylum Sarcodina, superclass Rhizopoda, class Lobosea, order Amoebida, family Entamoebidae, and genus *Entamoeba*.

The members of this genus are characterized by having a vesicular nucleus, a centrally (or near central) located small karyosome, and varying numbers of chromatin granules adhering to the nuclear membrane. These nuclear and other morphologic differences distinguish the species of *Entamoeba* except *E. histolytica*, *E. dispar*, and *E. moshkovskii* (previously known as the Laredo strain). The three said species are morphologically identical and of the same size. It was only recently that this *E. histolytica* species complex was resolved. Through isoenzyme analysis polymerase chain

reaction (PCR) restriction fragment length polymorphism (RFLP), and typing with monoclonal antibodies, these three species are now differentiated. *E. hartmanni*, formerly referred to as “small race” of *E. histolytica*, is differentiated primarily on the basis of size.

Parasite Biology

Entamoeba histolytica is a pseudopod-forming non-flagellated protozoan parasite. It is the most invasive of the *Entamoeba* parasites (which includes *E. dispar*, *E. moshkovskii*, *E. hartmanni*, *E. polecki*, *E. coli*, and *E. gingivalis*), and the only member of the family to cause colitis and liver abscess. The life cycle of *E. histolytica* consists of two stages: an infective cyst (Plate 2.1) and an invasive trophozoite form. No host other than humans is implicated in the life cycle,

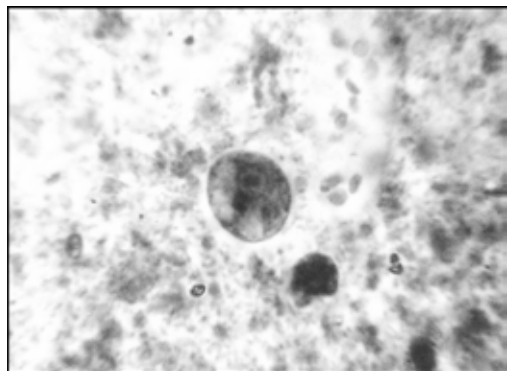


Plate 2.1. *Entamoeba histolytica* cyst (Courtesy of the Department of Parasitology, UP-CPH)

although natural infection of primates has been reported. The quadrinucleate cyst is resistant to gastric acidity and desiccation, and can survive in a moist environment for several weeks.

Infection with *E. histolytica* occurs when cysts are ingested from fecally-contaminated material (Figure 2.1). Other modes of transmission include venereal transmission through fecal-oral

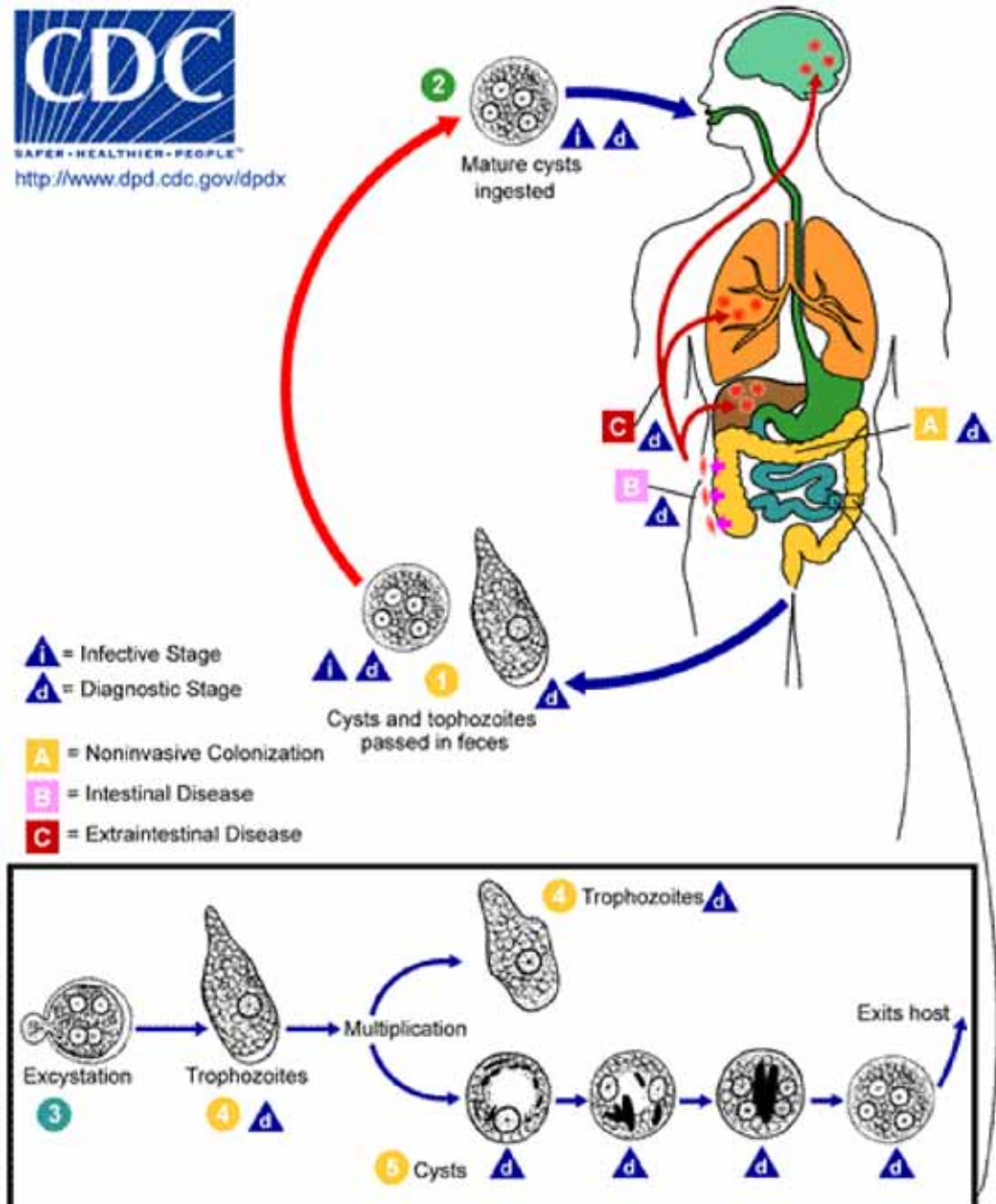


Figure 2.1. Life cycle of *Entamoeba histolytica*
(Accessed from www.dpd.cdc.gov/dpdx)

contact or direct colonic inoculation through contaminated enema equipment. Excystation occurs in the small or large bowel, where a cyst undergoes nuclear followed by cytoplasmic division to form eight trophozoites. The *E. histolytica* trophozoites are highly motile and possess pseudopodia (Plate 2.2). They vary in size from 12 to 60 μm in diameter (about 20 μm in average). Microscopic examination of fully-passed stool specimens reveals the characteristic progressive and directional movement of trophozoites, with pseudopodia as locomotory organelles. The hyaline pseudopodium is formed when the clear, glasslike ectoplasm, or outer layer is extruded, and the granular endoplasm flows into it. Ingested red blood cells are observed as pale, greenish, refractile bodies in the cytoplasm of the ameba. Cysts are usually spherical, and the size may vary from 10 to 20 μm . They are characterized by a highly refractile hyaline cyst wall, one to four nuclei, and rod-shaped (or cigar-shaped) chromatoidal bars. Trophozoites have the ability to colonize and/or invade the large bowel, while cysts are never found within invaded tissues. *E. histolytica* trophozoites multiply by binary fission. They encyst producing uninucleate cysts, which then undergo two successive nuclear divisions to form the characteristic quadrinucleate cysts (Plate 2.3).

E. histolytica is a eukaryotic organism but has several unusual features, including the lack of organelles that morphologically resemble mitochondria. Because nuclear-encoded mitochondrial genes such as *pyridine nucleotide transhydrogenase* and *hsp60* are present, *E. histolytica*, at one time may have contained mitochondria. There is no rough endoplasmic reticulum or Golgi apparatus, although cell surface and secreted proteins contain signal sequences, and tunicamycin inhibits protein glycosylation. Ribosomes form aggregated crystalline arrays in the cytoplasm of the trophozoite. Some differences in biochemical pathways from higher eukaryotes include the

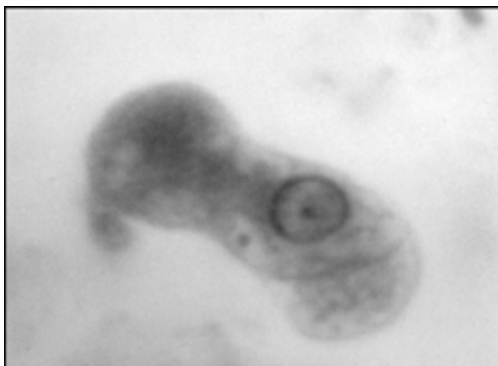


Plate 2.2. *Entamoeba histolytica* trophozoite (From World Health Organization. Bench Aids for the Diagnosis of Intestinal Parasites. Geneva: World Health Organization; 1994)

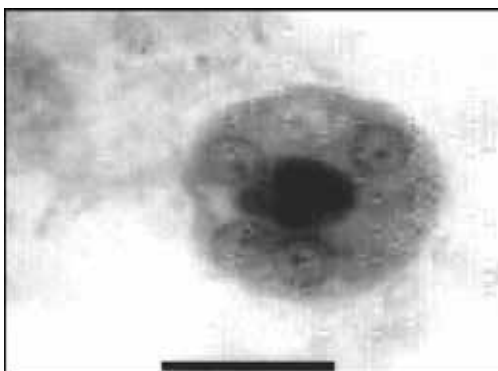


Plate 2.3. *Entamoeba histolytica* quadrinucleate cyst (From World Health Organization. Bench Aids for the Diagnosis of Intestinal Parasites. Geneva: World Health Organization; 1994)

lack of glutathione metabolism, the use of pyrophosphate instead of ATP at several steps in glycolysis, and the inability to synthesize purine nucleotides *de novo*. Glucose is actively transported into the cytoplasm, where the end products of carbohydrate metabolism are ethanol, carbon dioxide, and under aerobic conditions, acetate.

Pathogenesis and Clinical Manifestations

The proposed mechanisms for virulence are: production of enzymes or other cytotoxic substances, contact-dependent cell killing,

and cytophagocytosis. *In vitro*, amebic killing of target cultivated mammalian cells involve receptor-mediated adherence of ameba to target cells, amebic cytolysis of target cells, and amebic phagocytosis of killed or viable target cells. *E. histolytica* trophozoites adhere to the colonic mucosa through a galactose-inhibitable adherence lectin (Gal lectin). Then, the amebae kill mucosal cells by activation of their caspase-3, leading to their apoptotic death engulfment.

Recent studies have shown that susceptibility of humans to *E. histolytica* infection is associated with specific alleles of the *HLA complex*.

Majority of cases present as asymptomatic infections with cysts being passed out in the stools (cyst carrier state). The recent differentiation of *E. dispar* and *E. histolytica* by PCR has confirmed the high prevalence of non-pathogenic *E. dispar* compared to the pathogenic *E. histolytica*. However, studies also revealed that most *E. histolytica* infections in endemic communities are asymptomatic.

Amebic colitis clinically presents as gradual onset of abdominal pain and diarrhea with or without blood and mucus in the stools. Fever is not common and it occurs only in one third of patients. Although some patients may only have intermittent diarrhea alternating with constipation, children may develop fulminant colitis with severe bloody diarrhea, fever, and abdominal pain.

Ameboma occurs in less than 1% of intestinal infections. It clinically presents as a mass-like lesion with abdominal pain and a history of dysentery. It can be mistaken for carcinoma. Asymptomatic ameboma may also occur.

Amebic liver abscess (ALA) is the most common extra-intestinal form of amebiasis. The cardinal manifestations of ALA are fever and right upper quadrant (RUQ) pain. Several studies have shown these two as the most frequent complaints, particularly in acute cases (<2 weeks duration). In a Philippine

study involving 206 patients with probable ALA as diagnosed by ultrasound, the two most frequent manifestations were fever in 77% and RUQ pain in 83%. Pain is either localized in or referred to the right shoulder. The liver is tender, especially in acute cases, and hepatomegaly is present in 50% of cases. Chronic disease (>2 weeks duration) is found in older patients and it involves wasting with significant weight loss rather than fever. Only 30% of ALA cases have concurrent diarrhea. However, daily stool cultures revealed that 72% harbored trophozoites even in asymptomatic infections. Mortality in uncomplicated ALA is less than 1%.

The onset of amebic colitis may be sudden after an incubation period of 8 to 10 days, or after a long period of asymptomatic cyst carrier state. ALA may have all acute presentation of less than 2 weeks duration or a chronic one of more than 2 weeks duration. The recurrence rate was found to be 0.29% in a five-year study of ALA in Mexico.

The most serious complication of amebic colitis is perforation and secondary bacterial peritonitis. Colonic perforation occurs in 60% of fulminant colitis cases.

In ALA, the most serious complications are rupture into the pericardium with a mortality rate of 70%, rupture into the pleura with mortality of 15 to 30%, and super infection. Intraperitoneal rupture, which occurs in 2 to 7.5% of cases, is the second most common complication. However, it is not as serious as colonic perforation because ALA is sterile.

Secondary amebic meningoencephalitis occurs in 1 to 2%, and it should be considered in cases of amebiasis with abnormal mental status. Renal involvement caused by extension of ALA or retroperitoneal colonic perforation is rare. Genital involvement is caused by fistulae from ALA and colitis or primary infection through sexual transmission.

Natural or innate immunity to *E. histolytica* in the intestines involves mucin inhibition of

amebic attachment to the underlying mucosal cells. In the systemic circulation, the mechanism is that of complement-mediated killing of trophozoites. Acquired immunity primarily involves cell-mediated responses, although humoral responses may also contribute to anti-amebic immunity. Activated T-cells kill *E. histolytica* by: a) directly lysing trophozoites in a contact-dependent process; b) producing cytokines which activate macrophages and other effector cells (neutrophils and eosinophils); and c) providing helper effect for B-cell antibody production. *In vitro* studies using activated murine and human T-cells demonstrated significant killing of trophozoites in a contact-dependent and antibody independent manner. Cytokine studies revealed that interferon (IFN) and interleukin (IL-2) may have a role in activating macrophages for amebicidal activity. More recent studies demonstrated that activated macrophages produce nitric oxide (NO) which was lethal to trophozoites. Tumor necrosis factor (TNF) was shown to stimulate NO production. Although it is known that antibodies are produced against amebic antigens, there has been no direct evidence of T-cell help for B-cells. Studies have revealed that the principal antibody-dependent cell cytotoxicity (ADCC) did not work against amebae. Antibodies which were detected by seroepidemiologic studies and secretory IgA isolated in the gut may merely be an indicator of current or recent invasive amebiasis.

Amebic modulation of host immune responses exists. For instance, infected human subjects and animals have been shown to be in a state of immunosuppression during the acute stage of amebiasis. This state, characterized by T-cell hyporesponsiveness, suppressed proliferation and cytokine production, depressed delayed-type hypersensitivity (DTH), and macrophage suppression, is favorable for amebic survival. It is the reversal of these modulatory effects, which is the key in controlling amebiasis.

Acute amebic colitis should be differentiated from bacillary dysentery of the following etiology: *Shigella*, *Salmonella*, *Campylobacter*, *Yersinia*, and enteroinvasive *Escherichia coli* (Table 2.1). Although stools may be grossly bloody or heme-positive in both conditions, fever and significantly elevated leukocyte count are less common in amebic colitis. Another differential is inflammatory bowel disease. Amebic colitis should be ruled out before steroid therapy for inflammatory bowel disease is started because of the risk of developing toxic megacolon.

The differential diagnoses of ALA include pyogenic liver abscess, tuberculosis of the liver, and hepatic carcinoma. On the other hand, genital amebiasis should be differentiated from carcinoma, tuberculosis, chancroid, and lymphogranuloma venereum.

Table 2.1. Comparison of bacillary and amebic dysentery

Bacillary Dysentery	Amebic Dysentery
May be epidemic	Seldom epidemic
Acute onset	Gradual onset
Prodromal fever and malaise common	No prodromal features
Vomiting common	No vomiting
Patient prostrate	Patient usually ambulant
Watery, bloody diarrhea	Bloody diarrhea
Odorless stool	Fishy odor stool
Stool microscopy: numerous bacilli, pus cells,	
macrophages, red cells, no Charcot-Leyden crystals	Stool microscopy: few bacilli, red cells, trophozoites with ingested red blood cells, Charcot-Leyden crystals
Abdominal cramps common and severe	Mild abdominal cramps
Tenesmus common	Tenesmus uncommon
Natural history: spontaneous recovery in a few days, weeks or more; no relapse	Natural history: lasts for weeks; dysentery returns after remission; infection persists for years

Diagnosis

The standard method of parasitologic diagnosis is microscopic detection of the trophozoites and cysts in stool specimens. Ideally, a minimum of three stool specimens collected on different days should be examined. For detection of trophozoites, fresh stool specimens should be examined within 30 minutes from defecation. Using the direct fecal smear (DFS) with saline solution alone, the microscopist can observe trophozoite motility. Unidirectional movement is characteristic of *E. histolytica*. Using saline and methylene blue, *Entamoeba* species will stain blue, thus, differentiating them from white blood cells. Using saline and iodine, the nucleus and karyosome can be observed to differentiate *E. histolytica* from the non-pathogenic amebae (*E. hartmanni*, *E. coli*, *Endolimax nana*). The detection of *E. histolytica* trophozoites with ingested red blood cells is diagnostic of amebiasis. Charcot-Leyden crystals (Plate 2.4) can also be seen in the stool.

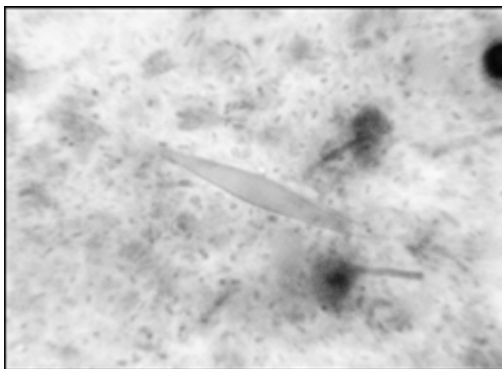


Plate 2.4. Charcot-Leyden crystal observed in stool specimen of a patient suffering from amebiasis (Courtesy of the Department of Parasitology, UP-CPH)

Concentration methods such as Formalin Ether/Ethyl Acetate Concentration Test (FECT) and Merthiolate Iodine Formalin Concentration Test (MIFC) are more sensitive than the DFS for detection of cysts. The

following morphologic structures are noted: size of the cyst, number of nuclei, location and appearance of the karyosome, the characteristic appearance of chromatoid bodies, and presence of cytoplasmic structures such as glycogen vacuole. *E. histolytica* can, thus, be differentiated from the non-pathogenic species, *E. hartmanni*, *E. coli*, *E. nana*, and *Iodameba bütschlii*. Stool culture using Robinson's and Inoki medium is more sensitive than stool microscopy, but is not routinely available.

Differentiation between *E. histolytica* and *E. dispar* is not possible by microscopy. This can only be done by PCR, enzyme-linked immunosorbent assay (ELISA), and isoenzyme analysis. The last is primarily a research technique. On the other hand, an ELISA-based assay for stool is now commercially available and studies have demonstrated a sensitivity of 80% and specificity of 99%. The use of PCR is limited by the requirement of sophisticated equipment. A Philippine study (n=497 stool samples) looked into the reliability of stool ELISA with PCR as gold standard (Plate 2.5). Sensitivity and specificity were 91% and 97%, respectively.

Detection of antibodies in the serum is still the key in the diagnosis of ALA. It must be noted that in ALA, microscopic detection cannot be done because aspiration is an invasive procedure, and trophozoites are missed because they are located in the periphery of the abscess.

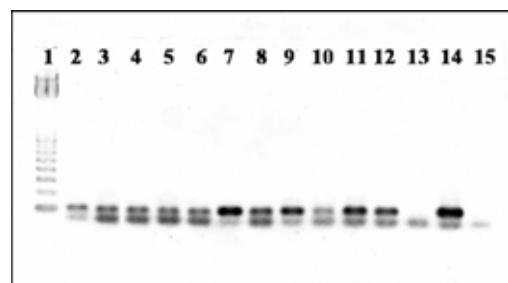


Plate 2.5. Agarose gel showing the 100bp PCR products of *Entamoeba histolytica*-positive stool specimens (lanes 2-15) (Courtesy of Dr. Windell Rivera)

To date, serological tests for amebic disease include indirect hemagglutination (IHAT), counter immunoelectrophoresis (CIE), agar gel diffusion (AGD), indirect fluorescent antibody test (IFAT), and ELISA. The IHAT can detect antibodies of a past infection even as long as 10 years ago. In contrast, the antibodies detected by ELISA, AGD, and CIE are of short duration, lasting for a few months. Antibodies have been demonstrated in asymptomatic intestinal infections so that serology can be used in the monitoring of a cyst carrier.

Ultrasound, computerized tomography (CT scan), and magnetic resonance imaging (MRI) are non-invasive and sensitive methods in early detection of ALA. Ultrasound (Plate 2.6) typically shows a round or oval hypoechoic area with wall echoes. In 80% of cases, this finding is seen in the right lobe of the liver. Multiple lesions occur in 50% of acute cases, and aspiration may be required to differentiate amebic from pyogenic abscess. Using serological methods (IHAT and IFAT) as gold standard, a Philippine study has shown that the sensitivity and specificity of ultrasound were 95% and 40%, respectively. However, as the results of the study still revealed some limitations in the use of ultrasound in the diagnosis of ALA, additional diagnostic ultrasound findings have yet to be identified.

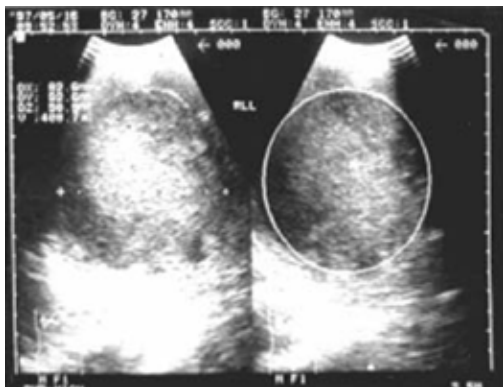


Plate 2.6. Ultrasound showing a solitary hypoechoic mass at the right lobe of the liver suggesting ALA (Courtesy of Dr. Pilarita Rivera)

Treatment and Prognosis

The treatment of amebiasis has two objectives: a) to cure invasive disease at both intestinal and extraintestinal sites; and b) to eliminate the passage of cysts from the intestinal lumen. Metronidazole is the drug of choice for the treatment of invasive amebiasis. Other 5-nitroimidazole derivatives such as tinidazole and secnidazole are also effective. Diloxanide furoate is the drug of choice for asymptomatic cyst passers. It is also given after a course of metronidazole for invasive amebiasis.

Percutaneous drainage of liver abscess is indicated for patients who do not respond to metronidazole and who need prompt symptomatic relief of severe pain. It is also done for those who have left lobe abscess that may rupture into the pericardium, large abscesses in danger of rupture, and multiple abscesses with a probable associated pyogenic etiology.

Epidemiology

For a long time, the species-complex referred to as *E. histolytica* was believed to infect 500 million people, or 10% of the world's population. However, with the recent redescription into three different species: the pathogenic *E. histolytica*, and the commensals, *E. dispar* and *E. moshkovskii*, the true prevalence of amebiasis is approximately 1 to 5% worldwide. There are 50 million *E. histolytica* infection cases, and 40,000 to 100,000 deaths due to amebiasis in the world per year. Thus, amebiasis is the third most important parasitic disease, after malaria and schistosomiasis, and second to malaria as the top cause of mortality among parasitic protozoans.

Humans are the major reservoirs of infection with *E. histolytica*. Ingestion of food and drink contaminated with *E. histolytica* cysts from human feces, and direct fecal-oral contact are the most common means of infection. Amebic infection is prevalent in the Indian subcontinent, Africa, East Asia, and South and Central America. In developing

countries, prevalence depends on the level of sanitation, crowding, socio-economic status, cultural habits, and age. In developed countries, infection is usually caused by *E. dispar*, and is prevalent in certain groups: immigrants, travelers from endemic countries, homosexual males (men having sex with men), HIV patients, and institutionalized people.

A microscopic study of diarrheic stools in Australia (n=5,921) revealed 177 (3%) positive samples. PCR detected 5 *E. histolytica*, 63 *E. dispar*, and 55 *E. moshkovskii* infections. The latter two species, which are both commensals, are 10 times more prevalent than *E. histolytica*. A stool survey done in Iran (n=16,592) showed 226 positive samples. Only 101 isolates were successfully cultured in Robinson's medium. Of these isolates, 93 (92.1%) were *E. dispar*, and only 8 (7.9%) were *E. histolytica* or mixed infections by PCR- RFLP.

A field study in Northern Philippines (n=1,872) showed 137 (7.3%) *E. dispar*, and 18 (0.96%) *E. histolytica* by PCR. A study in a mental institution (n=113) showed *E. histolytica* or *E. dispar* in 43 subjects (38.1%), while PCR detected 74 (65.5%) *E. histolytica*-positive samples, and 6 (5.3%) *E. dispar*/*E. histolytica* mixed samples.

Prevention and Control

The prevention and control of amebiasis depends on integrated and community-based efforts to improve environmental sanitation, and to provide for sanitary disposal of human feces, safe drinking water, and safe food. These efforts become more sustainable through health education and promotion. The proper use of latrines and practice of proper hygiene, such as washing of hands, should be emphasized. In communities where potable water is not available, drinking water should be boiled or filtered. Vegetables and fruits which are eaten raw should be thoroughly washed. The use of night soil for fertilizer should be avoided. Prompt diagnosis and treatment of amebiasis

cases should be done. Food handlers should be screened for cyst carriage, and asymptomatic cyst carriers should be treated.

Vaccines can be a cost-effective and potent strategy for amebiasis prevention and eradication. Unlike in other protozoan infections, amebic vaccine development has fewer problems. The ameba life cycle is simple, and no intermediate hosts are involved. Amebae are extracellularly located, and do not undergo antigenic variation. All these characteristics are supportive of an achievable amebic vaccine.

Studies have also demonstrated the acquisition of protective immunity to amebae, particularly that of mucosal immune response. Trials with recombinant amebic antigens as vaccines have proven to be more advantageous than inactivated/attenuated amebae. The candidate vaccine molecules which have been most intensely studied are the serine-rich *E. histolytica* protein (SREHP), the adherence lectin (Gal/GalNAc lectin), and the 29 kDa cysteine-rich amebic antigen. However, most of these studies have utilized animal models and artificial infection during challenge. Testing these candidate vaccines in humans and developing them as food-based vaccines will be in the forefront of future directions of amebiasis control.

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Commensal Amebae

Pilarita T. Rivera, Vicente Y. Belizario, Jr., Juan Antonio A. Solon

The presence of commensal amebae in the stools of an individual is significant for two reasons: (a) the amebae may be mistaken for the pathogenic *Entamoeba histolytica*; and (b) it is an indication of fecal contamination of food or water. Accurate identification of commensal amebae is therefore crucial.

Parasite Biology

Commensal amebae must be differentiated from pathogenic *E. histolytica* to avoid unnecessary treatment of patients infected with non-pathogenic species. The three genera of intestinal amebae can be differentiated through the morphological features of their nuclei. The genus *Entamoeba* has a spherical nucleus with a distinct nuclear membrane lined with chromatin granules and a small karyosome found near the center of the nucleus. Trophozoites usually have only one nucleus. The genus *Endolimax* has a vesicular nucleus with a relatively large, irregularly-shaped karyosome anchored to the nucleus by achromatic fibrils. The genus *Iodamoeba* is characterized by a large, chromatin-rich karyosome surrounded by a layer of achromatic globules and anchored to the nuclear membrane by achromatic fibrils.

All species have the following stages: trophozoite, precyst, cyst, and metacystic trophozoite; with the exception of *Entamoeba gingivalis*, which has no cyst stage, and does not inhabit the intestines. Humans are infected by commensal intestinal amebae through ingestion of viable cysts in food or water. Cysts pass through the acidic stomach and remain viable because of protective cyst walls. Excystation occurs in the alkaline environment of the lower small intestines. Metacystic trophozoites colonize the large intestines and live on the mucus coat covering the intestinal mucosa

(Figure 2.2). These amebae are non-invasive and do not cause disease.

Reproduction is by binary fission of the trophozoites. Encystation occurs as amebae pass through the lower colon where colonic contents are more dehydrated.

Entamoeba dispar

Entamoeba moshkovskii

Entamoeba dispar is morphologically similar to *E. histolytica*, but their DNA and ribosomal RNA are different. The former's isoenzyme pattern is different from that of *E. histolytica*.

Entamoeba moshkovskii isolates, although first detected in sewage, have been reported in some areas, such as North America, Italy, South Africa, Bangladesh, India, Iran, and Australia. It is a non-pathogenic species that is morphologically indistinguishable from *E. histolytica* and *E. dispar*, but differs from them biochemically and genetically. *E. moshkovskii* is also physiologically unique—it being osmotolerant, able to grow at room temperature (25–30°C optimum), able to survive at temperatures ranging from 0 to 41°C. It has limited pathogenicity in experimental trials in animals, but is non-pathogenic to humans. All human isolates have been found to belong to one group “ribodeme 2.”

Entamoeba hartmanni

The appearance of *E. hartmanni* is relatively similar to that of *E. histolytica* apart from its smaller size. Trophozoites of the former measure from 3 to 12 µm in diameter (compared to *E. histolytica* measuring 12–60 µm). Mature cysts measure 4 to 10 µm, are quadrinucleated like *E. histolytica*, and have rod-shaped chromatoid material with rounded or squared ends. Unlike

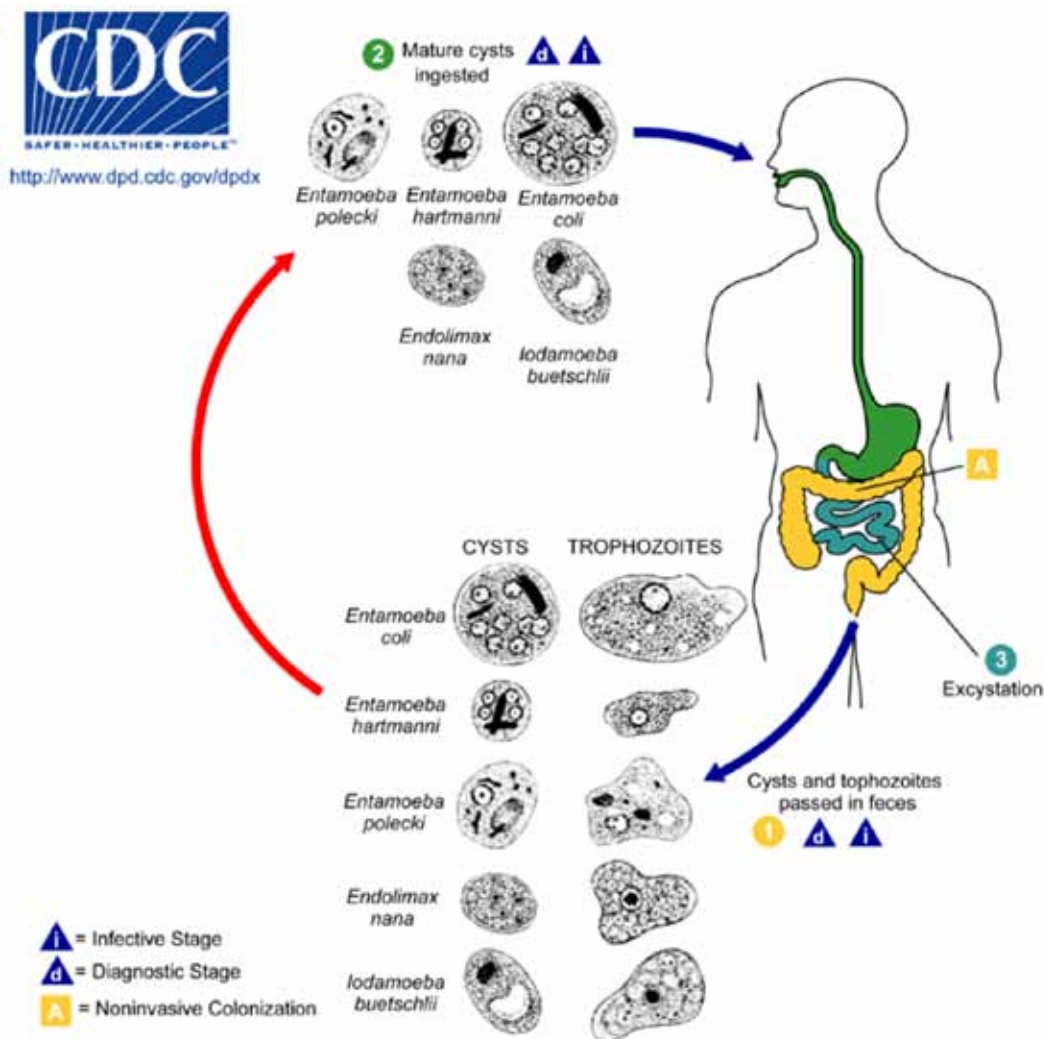


Figure 2.2. Life cycle of commensal amoebae
(Accessed from www.dpd.cdc.gov/dpdx)

E. histolytica, *E. hartmanni* does not ingest red blood cells.

Entamoeba coli

Entamoeba coli is cosmopolitan in distribution, and is considerably more common than other human amoebae. Trophozoites of *E. coli* measure 15 to 50 µm in diameter. It can be differentiated from *E. histolytica* trophozoite

by the following features: 1) a more vacuolated or granular endoplasm with bacteria and debris, but no red blood cells; 2) a narrower, less-differentiated ectoplasm; 3) broader and blunter pseudopodia used more for feeding than locomotion; 4) more sluggish, undirected movements; and 5) thicker, irregular peripheral chromatin with a large, eccentric karyosome in the nucleus (Plate 2.7).

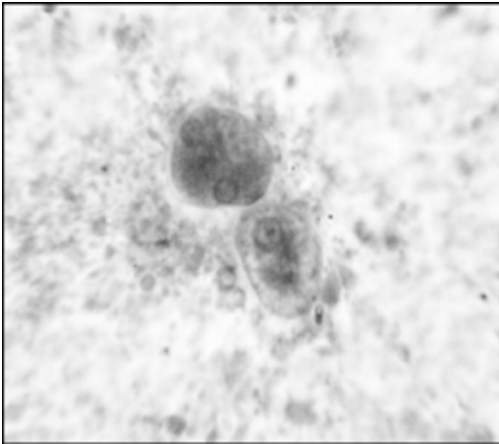


Plate 2.7. *Entamoeba coli* trophozoite (Courtesy of Department of Parasitology, UP-CPH)

An *E. coli* cyst may be differentiated from *E. histolytica* by: 1) its larger size (10 to 35 μm in diameter), 2) more nuclei (eight versus four in *E. histolytica*), 3) more granular cytoplasm, and 4) splinter-like chromatoidal bodies. Iodine staining reveals dark-staining, perinuclear masses, which are actually glycogen. Its location, surrounding the nucleus, is more characteristic of *E. coli* compared to *E. histolytica*.

Entamoeba polecki

Entamoeba chattoni

Entamoeba polecki is a parasite found in the intestines of pigs and monkeys. Rarely, it can infect humans, though a high prevalence (19%) was reported in some parts of Papua New Guinea (n=184 children). In these areas, both pig-to-human and human-to-human transmission may exist. Like *E. coli*, motility of trophozoites of *E. polecki* is sluggish. A small karyosome is centrally located in the nucleus. *E. polecki* can be distinguished from *E. histolytica* in that the former's cyst is consistently uninucleated, and chromatoidal bars are frequently angular or pointed. In stained fecal smears, the nuclear membrane and karyosome are very prominent.

Entamoeba chattoni, which is found in apes and monkeys, is morphologically identical to *E. polecki*. More recently, it has been detected in eight human infections. Identification of *E. chattoni* was done via isoenzyme analysis.

Entamoeba gingivalis

Entamoeba gingivalis can be found in the mouth. The trophozoite measures 10 to 20 μm . It moves quickly, and has numerous blunt pseudopodia. Food vacuoles that contain cellular debris (mostly leukocytes, which is characteristic of this species) and bacteria are numerous. *E. gingivalis* lives on the surface of gum and teeth, in gum pockets, and sometimes in the tonsillar crypts. They are abundant in cases of oral disease. This species has no cyst stage. Transmission is most probably direct: through kissing, droplet spray, or by sharing utensils.

Endolimax nana

Endolimax nana occurs with the same frequency as *Entamoeba coli*. Trophozoites are small, with a diameter of 5 to 12 μm , and exhibit sluggish movement. They have blunt, hyaline pseudopodia, and the nucleus has a large, irregular karyosome. Food vacuoles found in the cytoplasm may contain bacteria. Cysts measure about the same size as trophozoites, and are quadrinucleated when mature.

Iodamoeba bütschlii

The trophozoite averages 9 to 14 μm in diameter (ranging from 4-20 μm). It is identified by its characteristic large, vesicular nucleus with a large, central karyosome, surrounded by achromatic granules. There are no peripheral chromatin granules on the nuclear membrane. The cyst is about 9 to 10 μm in diameter (ranging from 6-16 μm), is uninucleated, and has a large glycogen body which stains dark brown with iodine (Plate 2.8).

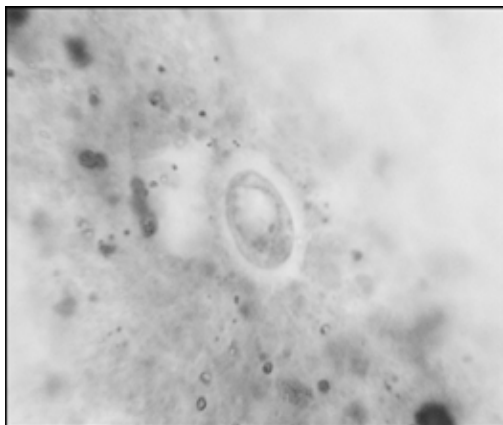


Plate 2.8. *Iodamoeba bütschlii* cyst
(Courtesy of the Department of Parasitology,
UP-CPH)

Diagnosis

Diagnosis is done through stool examination. Formalin ether/ethyl acetate concentration technique (FECT) and iodine stain are useful to differentiate the species. For *E. gingivalis*, a swab between the gums and teeth is examined for trophozoites. Cysts are recovered from formed stools, while trophozoites are recovered from watery or semi-formed stools. Trophozoites are best demonstrated by direct fecal smear. In recovering cysts, the use of concentration techniques like FECT and zinc sulfate flotation is useful.

Treatment

No treatment is necessary because these amebae do not cause disease.

Epidemiology

In single stool examinations of over 30,000 Filipinos, the prevalence of *Entamoeba coli* was about 21%, *Endolimax nana*, about 9%, and *Iodamoeba bütschlii*, 1%. Intestinal protozoan cysts were observed in 13.5% of overseas Filipino workers (OFWs) screened by the Department of Parasitology, UP Manila in

1998. A study on intestinal parasitic infections among food service workers in a tertiary hospital in Manila revealed that 20.3% were infected with *Endolimax nana* and 13.6% with *Entamoeba coli*. Another study of food handlers in selected school canteens in Manila showed infection rates of 22.8% for *Endolimax nana*, 17.9% for *Entamoeba coli*, and 0.8% each for *Entamoeba hartmanni* and *Iodamoeba bütschlii*.

Prevention and Control

Contraction of the organism may be prevented through proper disposal of human waste and good personal hygiene.

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Free-living Pathogenic Amebae

Edsel Maurice T. Salvana

Acanthamoeba spp.

Parasite Biology

Acanthamoeba is a ubiquitous, free-living ameba that is the etiologic agent of *Acanthamoeba* keratitis (AK) and granulomatous amebic encephalitis (GAE). *Acanthamoeba* is characterized by an active trophozoite stage with characteristic prominent “thorn-like” appendages (acanthopodia); and a highly resilient cyst stage into which it transforms when environmental conditions are not favorable. It is an aquatic organism that is found in a myriad of natural and artificial environments, and can survive even in contact lens cleaning solutions. Motile trophozoites feed on gram-negative bacteria, blue-green algae, or yeasts and reproduce by binary fission, but can also adapt to feed on corneal epithelial cells and neurologic tissue through phagocytosis and secretion of lytic enzymes.

Morphologically, *Acanthamoeba* trophozoites exhibit a characteristic single large nucleus with a centrally-located, densely staining nucleolus; a large endosome; finely granulated cytoplasm; and a large contractile vacuole. Small, spiny filaments for locomotion known as acanthopodia are evident on phase-contrast microscopy (Plate 2.9).

Acanthamoeba has only two stages, cysts and trophozoites, in its life cycle. No flagellated stage exists as part of the life cycle. The trophozoites replicate by mitosis (nuclear membrane does not remain intact). The trophozoites are the infective stage, although both cysts and trophozoites gain entry into the body through various means. Entry can occur through the eye, the nasal passages to the lower respiratory tract, or ulcerated or broken skin (Figure 2.3).

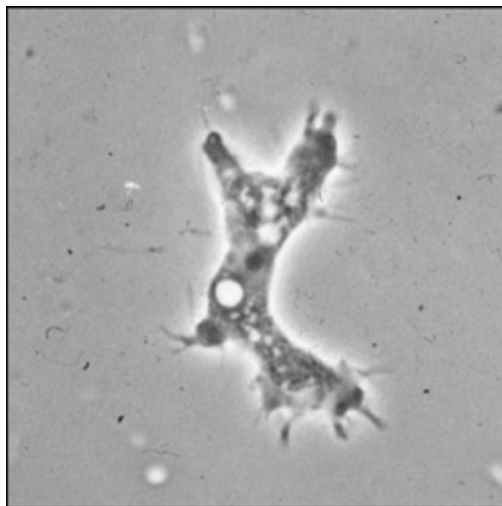


Plate 2.9. *Acanthamoeba* trophozoite exhibiting characteristic acanthopodia (Accessed from www.dpd.cdc.gov/dpdx)

The presence of naturally-occurring bacterial endosymbionts in *Acanthamoeba* spp. has been reported. Although the presence of bacterial symbionts is widespread among small, free-living amebae, the significance of this association is not known. Recently, *Acanthamoeba* spp. have been implicated as possible reservoir hosts for medically important bacteria such as *Legionella* spp., mycobacteria, and gram-negative bacilli such as *E. coli*.

Pathogenesis and Clinical Manifestations

A. *Acanthamoeba* Keratitis

Acanthamoeba was first described as an opportunistic ocular surface pathogen causing keratitis in 1974. AK is associated with the use of improperly disinfected soft contact lenses, particularly those which are rinsed with tap water or contaminated lens solution. An immunocompromised state contributes to

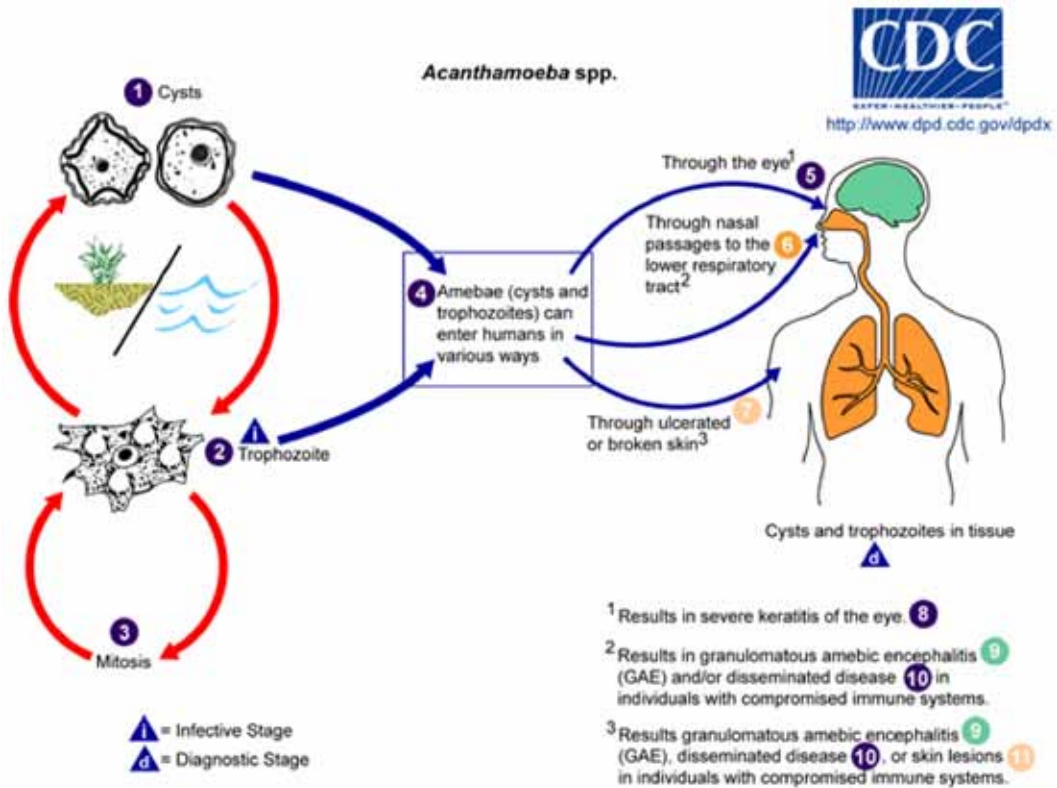


Figure 2.3. Life cycle of *Acanthamoeba* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

increased susceptibility to infection, and may lead to disseminated disease in the lungs and brain (GAE).

Symptoms of AK include severe ocular pain and blurring of vision. Corneal ulceration with progressive corneal infiltration may occur. Primary amebic infection or secondary bacterial infection may lead to hypopyon formation. Progression of infection may cause scleritis and iritis, and may ultimately lead to vision loss. Major differentials which need to be ruled out include fungal and herpetic keratitis.

B. *Granulomatous Amebic Encephalitis*

Acanthamoeba was documented as the causative agent of human GAE by Stamm in 1972. Amebae were demonstrated in brain sections of a GAE patient using indirect

fluorescence microscopy. GAE usually occurs in immunocompromised hosts including the chronically ill and debilitated, and those on immunosuppressive agents such as chemotherapy and anti-rejection medications. The acquired immune deficiency syndrome (AIDS) epidemic in the 1980's dramatically increased the numbers of person with GAE, but these numbers have since fallen with the advent of highly effective antiretroviral therapy.

Signs and symptoms of GAE are generally related to destruction of brain tissue and the associated meningeal irritation. Systemic manifestations early in the course include fever, malaise, and anorexia. Neurologic symptoms may include increased sleeping time, severe headache, mental status changes, epilepsy, and coma. Neurologic findings depending on the

location of the lesions include hemiparesis, blurring of vision, diplopia, cranial nerve deficits, ataxia, and increased intracranial pressure.

Entry of *Acanthamoeba* into the central nervous system is still incompletely understood. From a primary site of infection in the skin or lungs, the likely route of invasion is hematogenous. Direct infection through the olfactory valves has also been proposed, but not conclusively demonstrated. Recent reviews have focused on blood-borne invasion, with a combination of host factors, elucidation of serine proteases, and parasite adhesion using a mannose-binding protein all contributing to brain endothelial cell damage and subsequent breakdown of the blood-brain barrier.

Gross examination of neural tissue post-mortem reveals cerebral hemispheres that are edematous and soft, with areas of hemorrhage and focal abscesses. The most affected areas of the brain are the posterior fossa structures, thalamus, and the brainstem. In the affected areas, the leptomeninges are opaque and exhibit purulent exudates and vascular congestion.

The incubation period from initial inoculation is approximately 10 days, with a subacute and chronic clinical course of infection that lasts for several weeks to several months. The clinical manifestations of disease include decreased sensorium, altered mental status, meningitis, and neurologic deficits. The natural course of the disease eventually results in coma and death.

Diagnosis

Acanthamoeba keratitis is diagnosed by epithelial biopsy or corneal scrapings for recoverable ameba with characteristic staining patterns on histologic analysis. Amebae have also been isolated from the contact lens and lens solution of patients. Species-specific identification can be made from culture and molecular analysis through PCR. Known species that have caused AK include *A. castellani*, *A. culbertsoni*, *A. hutchetti*, *A. polyphaga*, and *A. rhysoides*.

Diagnosis of GAE is usually made post-mortem in most cases. The rarity of the disease and unfamiliarity of most physicians with the pathogen contribute to frequently missed diagnosis. Signs and symptoms of disease are usually attributed to more common differentials. Moreover, recovery of ameba from cerebrospinal fluid is exceedingly rare, and imaging results are generally nonspecific.

Immunocompromised patients such as those with AIDS are at the highest risk for acquiring GAE. While opportunistic infections of the central nervous system such as *Cryptococcus* meningitis and toxoplasmosis are much more common than GAE, the lack of response despite appropriate treatment should prompt a more thorough evaluation for more esoteric organisms.

Specific diagnosis depends on demonstrating the trophozoites or cysts in tissues using histopathologic stains and microscopy. The organisms can rarely be demonstrated in the cerebrospinal fluid and can be cultured for further studies.

Treatment

Medical treatment of AK has been met with increasing success in recent years. While historically, only surgical excision of the infected cornea with subsequent corneal transplantation was curative, early recognition of AK coupled with aggressive combination anti-amebic agents can preclude the need for extensive surgery. D'Aversa and his colleagues have achieved acceptable results with clotrimazole combined with pentamidine, isethionate, and neosporin. Other agents that have been used include polyhexamethylene biguanide, propamidine, dibromopropamidine isethionate, neomycin, paromomycin, polymyxin B, ketoconazole, miconazole, and itraconazole. Topical corticosteroids should be avoided, as this retards the immune response. Advanced AK usually requires debridement, but complete excision of the cornea can be avoided if the infection is confined to more superficial areas.

Deep lamellar keratectomy is the procedure of choice.

Clinically apparent neurologic disease in GAE usually heralds a fatal outcome within 3 to 40 days. A few patients have shown good responses to combinations of amphotericin B, pentamidine isethionate, sulfadiazine, flucytosine, fluconazole or itraconazole. One liver transplant patient survived after decompressive frontal lobectomy and treatment with amphotericin, cotrimoxazole, and rifampin. Poor prognostic factors include severe immunosuppression and advanced disease.

Epidemiology

Acanthamoeba spp. have a protean distribution, having been isolated from a multitude of natural and artificial aquatic environments including fresh and salt water, sewage, hospital equipment, and contact lenses and lens solution.

De Jonckheere first diagnosed *Acanthamoeba* GAE in a living patient in 1991. Previously, diagnosis of GAE was post-mortem. AK was recognized earlier in the 1970s and has been reported in the United States, Europe, South America, and Asia. The first case of AK was recognized in the Philippines in the 1990s from a patient from the Philippine General Hospital, and samples obtained from the patient was shown to cause GAE in mice. Multiple environmental isolates have likewise been well-characterized from all over the Philippines, including a few containing endosymbionts.

Prevention and Control

The ubiquitous nature of *Acanthamoeba* spp. makes exposure unavoidable. A robust immune system is able to prevent infection, except in relatively immunocompromised sites such as the cornea. Meticulous contact lens hygiene is essential in avoiding infection, and rinsing contact lenses in tap water should be avoided. Prolonged heating and boiling kill amebic trophozoites and cyst forms. Immunocompromised persons should be aware

of the risk of infection, and physicians treating these patients should maintain a high index of suspicion in the presence of compatible signs and symptoms of infection which do not respond to conventional antimicrobial therapy.

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Naegleria spp.

Parasite Biology

Naegleria spp. are free-living protozoans with two vegetative forms: an ameba (trophozoite form), and a flagellate (swimming form). A dormant cyst form is produced when conditions are not favorable. Transformation from the trophozoite to the flagellate form may facilitate more rapid movement toward food sources.

There are two forms of trophozoites of *Naegleria fowleri*: ameboid and ameboflagellate, only the former of which is found in humans (Plate 2.10). The ameboid trophozoites measure

10 to 35 μm but when rounded are usually 10 to 15 μm in diameter. In culture, trophozoites may get over 40 μm . The cytoplasm is granular and contains many vacuoles. The single nucleus is large and has a large, dense karyosome and lacks peripheral chromatin.

Naegleria spp. are thermophilic organisms which thrive best in hot springs and other warm aquatic environments. Both nonpathogenic and pathogenic forms exist. Only *Naegleria fowleri* has been reported to consistently cause disease in humans, although some non-*fowleri* species may cause opportunistic infections.

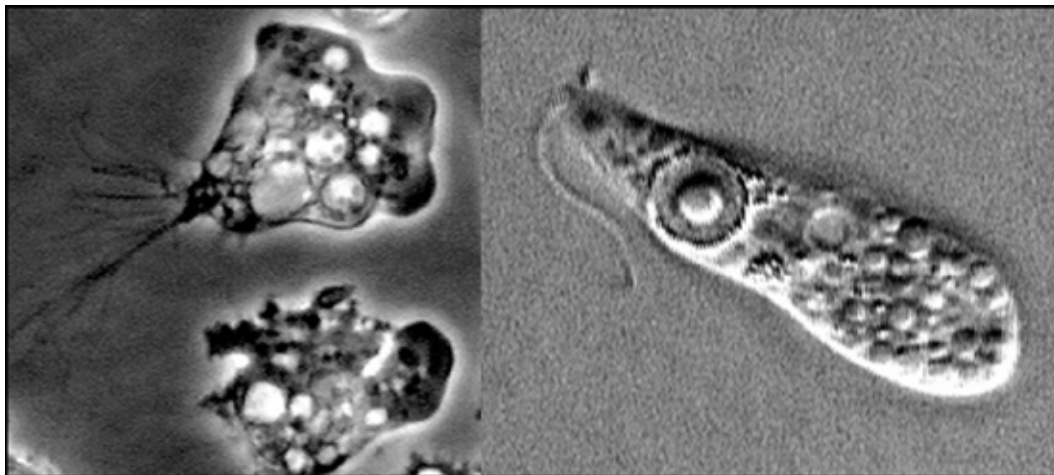


Plate 2.10. *Naegleria fowleri* trophozoites in ameboid (left) and ameboflagellate (right) forms
(Accessed from www.dpd.cdc.gov/dpdx)

Naegleria fowleri has three stages, cysts, trophozoites, and flagellated forms, in its life cycle. The trophozoites replicate by promitosis (nuclear membrane remains intact) and can turn into temporary non-feeding flagellated forms, which usually revert back to the trophozoite stage. Trophozoites infect humans or animals

by penetrating the nasal mucosa and migrating to the brain via the olfactory nerves. *N. fowleri* trophozoites are found in cerebrospinal fluid (CSF) and tissue, while flagellated forms are occasionally found in CSF. Cysts are not seen in brain tissue (Figure 2.4).

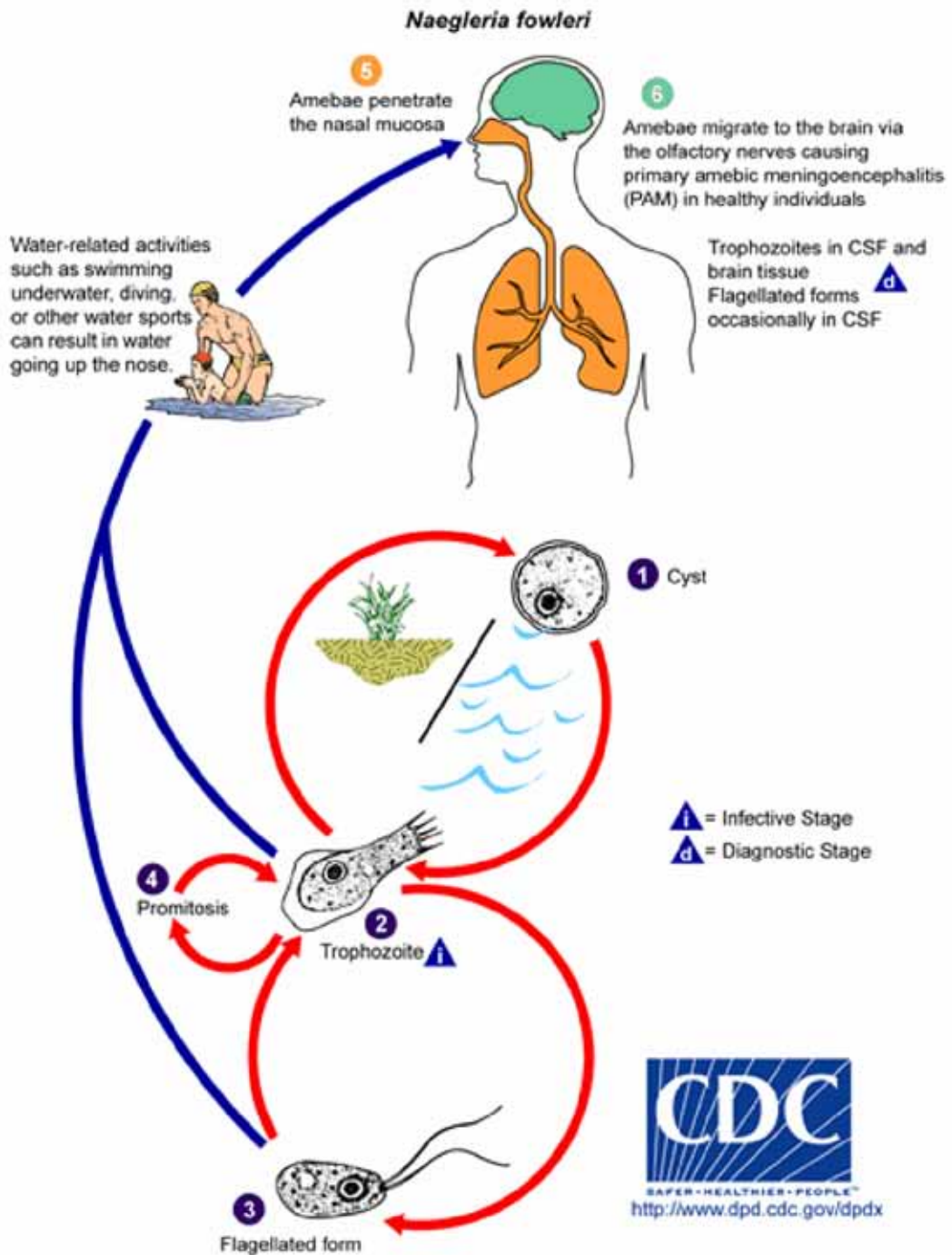


Figure 2.4. Life cycle of *Naegleria fowleri*
 (Accessed from www.dpd.cdc.gov/dpdx)

Pathogenesis and Clinical Manifestations

N. fowleri is the causative agent of a rare but rapidly destructive and fatal meningoencephalitis termed primary amebic meningoencephalitis (PAM). In contrast to GAE which is predominantly an opportunistic infection, PAM usually occurs in previously healthy adults with a history of swimming. Therefore, in contrast to *Acanthamoeba* which is largely an opportunistic organism, *N. fowleri* is considered a true pathogen.

N. fowleri is able to survive in elevated temperatures and reproduces rapidly in temperatures above 30°C. Aside from naturally occurring hot springs, warm geothermal plant effluent into lakes and streams can lead to proliferation of amebae.

Most cases of PAM have occurred in young, healthy persons who swim in contaminated water. The route of entry is through invasion of organisms through the olfactory bulb after accidental inhalation of water containing the organisms. The sustentacular cells of the olfactory neuroepithelium are thought to phagocytose the amebae and transport these through the cribriform plate and into the brain. Multiple mechanisms then come into play, producing a cytopathic effect on host tissues. These mechanisms include secretion of lytic enzymes, membrane pore-forming proteins, factors which induce apoptosis, and direct feeding on cells by the amebae.

In humans, PAM presents as fever, nausea, vomiting, headache, nuchal rigidity, and mental status changes, with rapid progression to coma and death. Characteristic cerebrospinal fluid findings include elevated white blood cell count with neutrophilic predominance, high protein, and low glucose.

Post-mortem examination of infected brain shows hemorrhagic necrosis, particularly of the olfactory bulbs, congestion and edema of neural tissue. Leptomeninges are inflamed and congested as well. Microscopic examination

shows a fibrinopurulent exudate consisting mostly of neutrophils in the leptomeninges and brain tissue, and pockets of amebae with scant inflammatory exudates in necrotic areas. Death usually occurs as a result of cerebral or cerebellar herniation as a result of increased intracranial pressure.

Diagnosis

Diagnosis of PAM is usually suspected in persons with a compatible history of exposure and a rapidly progressive meningoencephalitis. In the past, definitive diagnosis of PAM was based on demonstration of characteristic trophozoites in the brain and cerebrospinal fluid. Aspirates from suspected infections, when introduced into bacteria-seeded agar culture medium, will exhibit active trophozoites within 24 hours.

Naegleria trophozoites can be identified by the presence of blunt, lobose pseudopodia and directional motility. Flagellation tests have poor sensitivity for identification since amebae which test negative have been subsequently identified as *Naegleria* spp. and *Naegleria fowleri* with more sensitive and specific molecular techniques such as PCR and immunostaining. Serology utilizing ELISA is less useful in diagnosing active infection since healthy individuals especially in endemic areas have been shown to have positive antibody titers.

Treatment

Most persons infected with *Naegleria* die prior to institution of effective treatment. Symptoms of PAM are indistinguishable from bacterial meningitis. Initial CSF results are suggestive of a bacterial etiology, and so patients are typically treated with antibiotics which have no activity against *Naegleria*.

Amphotericin B in combination with clotrimazole is synergistic, and has been successfully used to treat PAM. Amphotericin B produces deleterious changes in the nucleus

and mitochondria of the ameba, decreases the number of food vacuoles, and increases the formation of autophagic vacuoles. Ameba exposed to amphotericin B exhibit decreased pseudopod formation and form blebs on the plasma membrane. Newer agents such as azithromycin and voriconazole have been shown to be active against *N. fowleri*, both *in vitro* and *in vivo*.

Epidemiology

Distribution of *Naegleria* in freshwater lakes and ponds has been correlated with physical, chemical, and biological parameters. Strains have been frequently isolated from thermal effluents, hot springs, and water with naturally or artificially elevated temperatures. Fecal coliform contamination provides a ready food source for ameba, and may increase the risk of infection due to higher density of organisms.

Studies on local *Naegleria* have identified a new species which is morphologically indistinguishable but biochemically distinct from other known species. Isolates from a thermally-polluted stream, an artificially-heated swimming pool, and from the brain aspirate of a young patient have all yielded a single species, *N. philippinensis*. This has been extensively studied by Castro et al., and Matias et al. Only one case of PAM has been reported locally, in a young male with a history of swimming in fresh water. He responded well to amphotericin B infusion.

Two Philippine isolates of *Naegleria* (NSzu and RITM strains) have been evaluated for pathogenicity. Massive doses of amebae successfully established infection in the brain and caused death in some mice within two to six days post-inoculation. Clinical features of infection and histopathology were compatible with PAM.

Prevention and Control

The ubiquitous nature of *Naegleria*, in contrast to the rarity of infection seems to indicate that incidental exposure is unlikely to lead to disease. Most instances of infection are related to invasion of the ameba through the olfactory bulbs, and so avoiding immersion of the head and accidental inhalation of water should be practiced in endemic areas and in hot springs. No known cases of PAM have resulted from drinking ameba-infected water.

Naegleria fowleri is easily killed by chlorination of water at 1 ppm or higher. Infection has been reported from swimming in contaminated water with inadequate chlorination, and so recommendations for appropriate decontamination of swimming water should be followed, especially in areas of high prevalence.

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Ciliates and Flagellates

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes

Balantidium coli

Initially identified as *Paramecium coli* by Malmsten in 1857, *Balantidium coli* was later described and placed under a separate genus in 1863. *B. coli* is the causative agent of the zoonotic disease called balantidiasis, balantidiosis, or balantidial dysentery. It is considered as the largest protozoan parasite affecting humans and is the only ciliate known to cause human disease. It is capable of attacking the intestinal epithelium, resulting in ulcer formation which, in turn, causes bloody diarrhea similar to that of amebic dysentery. This organism is primarily associated with pigs, its normal host.

Parasite Biology

Balantidium coli trophozoite measures 30 to 150 μm long and 25 to 120 μm wide. For locomotion, trophozoites are covered with cilia arranged in a longitudinal pattern extending from the oral to the caudal region. It has a cytostome, an oral apparatus at the tapered anterior end, through which it acquires food, and a cytopyge at the rounded posterior end through which it excretes waste. It has two dissimilar nuclei. The macronucleus is usually bean-shaped and can easily be identified in stained specimens, while the micronucleus is round and lies in the concavity of the macronucleus. *B. coli* has two contractile vacuoles that act as osmoregulatory organelles. The parasite also contains extrusive organelles called mucocysts which are located beneath the cell membrane.

B. coli cysts are spherical to slightly ovoid in shape and measure 40 to 60 μm in diameter. They are covered with thick cell walls (double-walled). Unlike amebae, encystation does not result in an increase in number of nuclei.

Human infection results from ingestion of food and/or water contaminated with *B. coli* cysts. The incubation period is normally from 4 to 5 days. Ingested cysts excyst in the small intestines and become trophozoites. Trophozoites inhabit the lumen, mucosa, and submucosa of the large intestines, primarily the cecal region. They cause pathologic changes in the colonic wall and mucosa. Parasite reproduction occurs asexually through asymmetric binary fission, although sexual reproduction through conjugation has been reported. Cysts are formed principally as protection for survival outside the host. The parasites encyst during intestinal transport or after evacuation of semi-formed stools. Cysts are the infective stage, and they may remain viable for several weeks (Figure 2.5).

Pathogenesis and Clinical Manifestations

Balantidium coli trophozoites are capable of attacking the intestinal epithelium and creating a characteristic ulcer with a rounded base and wide neck, in contrast to the flask-shaped, narrow necked ulcers of amebiasis. Ulceration is caused by the lytic enzyme hyaluronidase which is secreted by the trophozoite. The trophozoites are abundant in exudates on mucosal surfaces; while inflammatory cells and trophozoites are numerous in the base of the ulcers. Trophozoites also invade the submucosa and the muscular coat, including blood vessels and lymphatics.

Intrinsic host factors including nutritional status, intestinal bacteria flora, achlorhydria, alcoholism, and presence of chronic disease contribute to host susceptibility to and severity of *B. coli* infection. It has been suggested by some investigators that *B. coli* mucocysts might have a function in the adhesion of parasitic ciliates that may contribute to parasite virulence, although no definitive study has proven this. In

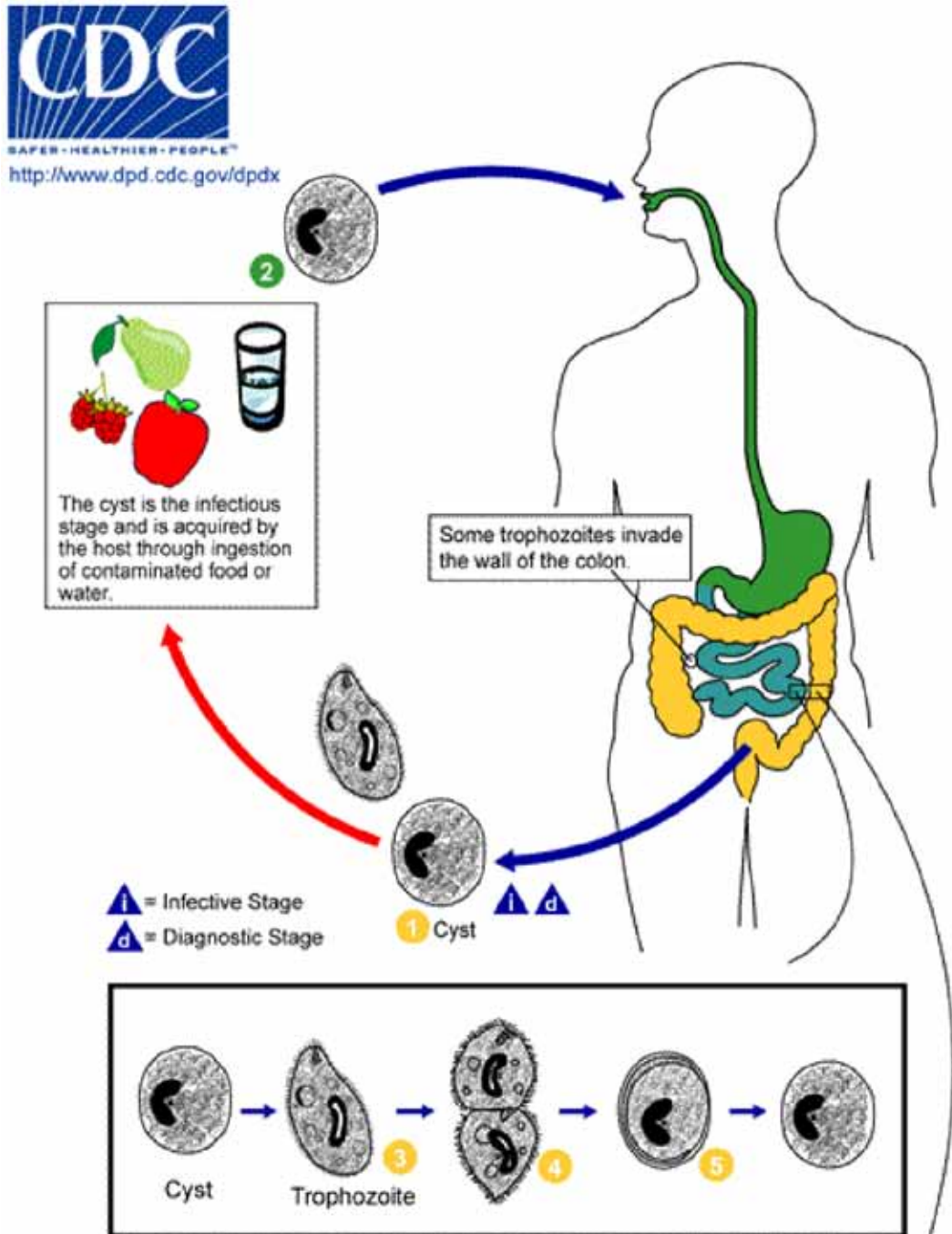


Figure 2.5. Life cycle of *Balantidium coli*
(Accessed from www.dpd.cdc.gov/dpdx)

one study, it was shown that mucocysts in *B. coli* trophozoites obtained from symptomatic pigs were more numerous compared with trophozoites obtained from asymptomatic hosts. In addition, co-infection with other organisms may also contribute to severity of *B. coli* infection. The presence of *Salmonella* in the intestines has been shown to aggravate balantidiasis by invading the ulcers caused by the protozoan.

Balantidiasis has three forms of clinical manifestations. Asymptomatic carriers are those who do not present with diarrhea or dysentery, but may serve as parasite reservoir in the community. Fulminant balantidiasis, or balantidial dysentery involves diarrhea with bloody and mucoid stools, which is sometimes indistinguishable from amebic dysentery. Acute cases may have 6 to 15 episodes of diarrhea per day accompanied by abdominal pain, nausea, and vomiting. This form of balantidiasis is often associated with immunocompromised and malnourished states. The third form of balantidiasis is the chronic form wherein diarrhea may alternate with constipation, and may be accompanied by nonspecific symptoms such as abdominal pain or cramping, anemia, and cachexia.

B. coli can spread to extraintestinal sites including the mesenteric nodes, appendix, liver, genitourinary sites, pleura, and lungs. One case report involved the detection of a cavitary lesion in the right upper lobe of the lung on chest radiograph in a patient who presented with hemoptysis. The patient had a history of insulin-dependent diabetes and organic farming using pig manure as fertilizer. Bronchoalveolar lavage revealed *B. coli* trophozoites. Another case presented with pulmonary hemorrhage and iron deficiency anemia, and revealed numerous *B. coli* trophozoites by bronchial biopsy and lavage.

Complications of balantidiasis include intestinal perforation and acute appendicitis. Cases of mortality related to balantidiasis were reported to be associated with intestinal hemorrhage and shock, intestinal perforation, or sepsis.

Diagnosis

Diagnosis is made by microscopic demonstration of trophozoites and cysts in feces using direct examination or concentration (sedimentation or flotation) techniques. Repeated stool examinations may be done to increase sensitivity. Demonstrating the presence of trophozoites in biopsy specimens from lesions obtained through sigmoidoscopy is likewise diagnostic. Bronchoalveolar washings may also contain *B. coli* trophozoites in the case of pulmonary infection.

Treatment

The treatment of choice for balantidiasis is tetracycline or metronidazole. Treatment in adults and older children is with tetracycline 500 mg or 40 mg/kg/dose divided in four doses for 10 days. Tetracycline is contraindicated in children less than eight years of age and in pregnant women. Metronidazole 750 mg three times daily, or 35 to 50 mg/kg body weight/day in three divided doses, may be given for 5 days. Iodoquinol may also be given at 650 mg, or 40 mg/kg/dose, divided in three doses for 20 days. Other alternative treatments for balantidiasis include doxycycline and nitazoxanide. Currently there are no reports of *B. coli* exhibiting drug resistance.

Epidemiology

The distribution of *B. coli* is cosmopolitan and is more prevalent in areas with poor sanitation, close contact with pigs or pig feces (e.g., farms, abattoirs), and in overcrowded institutions (e.g., asylum, orphanages, prisons). Warm and humid climates in tropical and subtropical countries can also contribute to the survival of cysts. High prevalence levels in pigs have been reported in regions in Latin America and the Middle East, as well as in the Philippines, Papua New Guinea, and the West Irian province of Indonesia.

There is an estimated 1% worldwide prevalence of human *B. coli* infection. Pigs

are the major host of balantidiasis, although primates have been reported to harbor infection. Prevalence studies in the United States and in Europe have reported infection rates ranging from 5% to as high as 100% in some areas. In a study done in two (northern and southern) sites in the Philippines, an examination of pigs revealed 66.1% prevalence of *B. coli* infection. There has been a single report of an outbreak of balantidiasis that occurred in the Truk island in Micronesia in 1971.

Prevention and Control

Control measures for balantidiasis include proper sanitation, safe water supply, good personal hygiene, and protection of food from contamination. Measures to limit contact of pigs with water sources and food crops may also contribute to reducing transmission and infection. Use of pig feces as fertilizer should also be avoided. Though cysts may be resistant to environmental conditions and may survive for long periods of time, they are easily inactivated by heat and by 1% sodium hypochlorite. Ordinary chlorination of water is not effective against *B. coli* cysts.

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Giardia duodenalis

Juan Antonio A. Solon

Giardia duodenalis is an intestinal parasitic flagellate of worldwide distribution. It is known to cause epidemic and endemic diarrhea. This protozoan is also known as *Giardia intestinalis* or *G. lamblia*. It was first discovered in 1681 by Antoine van Leeuwenhoek in his own stools and was first described by Lambl in 1859 who called it *Cercomonas intestinalis*. It was later renamed *Giardia lamblia* by Stiles in 1915. The disease caused by this parasite is called giardiasis, and this manifests as a significant but not life-threatening gastrointestinal disease.

Parasite Biology

Giardia duodenalis is a flagellate that lives in the duodenum, jejunum, and upper ileum of humans. It has a simple asexual life cycle that includes trophozoites and quadrinucleated infective cyst stages. Molecular typing of isolates shows that those which parasitize humans can be classified as belonging to either assemblage A or B genotypes based on specific sequences in the small subunit of their ribosomal RNA.

The trophozoites measure 9 to 12 μm long by 5 to 15 μm wide. They are pyriform or teardrop shaped, pointed posteriorly, with a pair of ovoidal nuclei, one on each side of the midline. The dorsal side of the organism is convex, while the ventral side is concave with a large adhesive disc used for attachment. It is bilaterally symmetrical, with a distinct medial line called the axostyle. The parasite is propelled into an erratic tumbling motion by four pairs of flagella arising from superficial organelles in the ventral side of the body. Trophozoites divide by longitudinal binary fission and are found in diarrheic stools. Antigenic variation results in the entire surface of the parasite being covered with variant-specific surface proteins (VSPs).

Cysts are ovoid and measure 8 to 12 μm long by 7 to 10 μm wide. The young cysts have two nuclei, while the mature cysts have four. Cysts are characterized by flagella retracted into axonemes, the median or parabasal body, and deeply stained curved fibrils surrounded by a tough hyaline cyst wall secreted from condensed cytoplasm.

Cysts from animals or human feces are transferred to the mouth via contaminated hands, food, or water. Once mature cysts (infective stage) are ingested, they pass safely through the stomach and excyst in the duodenum (in about 30 minutes) developing into trophozoites which rapidly multiply and attach to the intestinal villi causing pathologic changes. The trophozoites may then be found in the jejunum. As the feces enters the colon and dehydrates, the parasite then encysts. After encystment, mature cysts are passed out in the feces and are infectious (Figure 2.6).

Pathogenesis and Clinical Manifestations

Infection with *G. duodenalis* occurs when the host ingests food or water contaminated with the mature cysts. Depending on the strain involved, infection can occur with one ingesting as few as 10 cysts. The ability of the parasite to cause disease can be traced to its ability to alter mucosal intestinal cells once it has attached to the apical portion of the enterocyte. The parasite attaches to the intestinal cells via an adhesive sucking disc located on its ventral side, causing mechanical irritation in the affected tissues. Several studies have investigated this mechanism of attachment. In monolayer studies, it was noted that attachment was influenced by certain physical factors such as temperature and pH. Attachment was observed to be maximal at

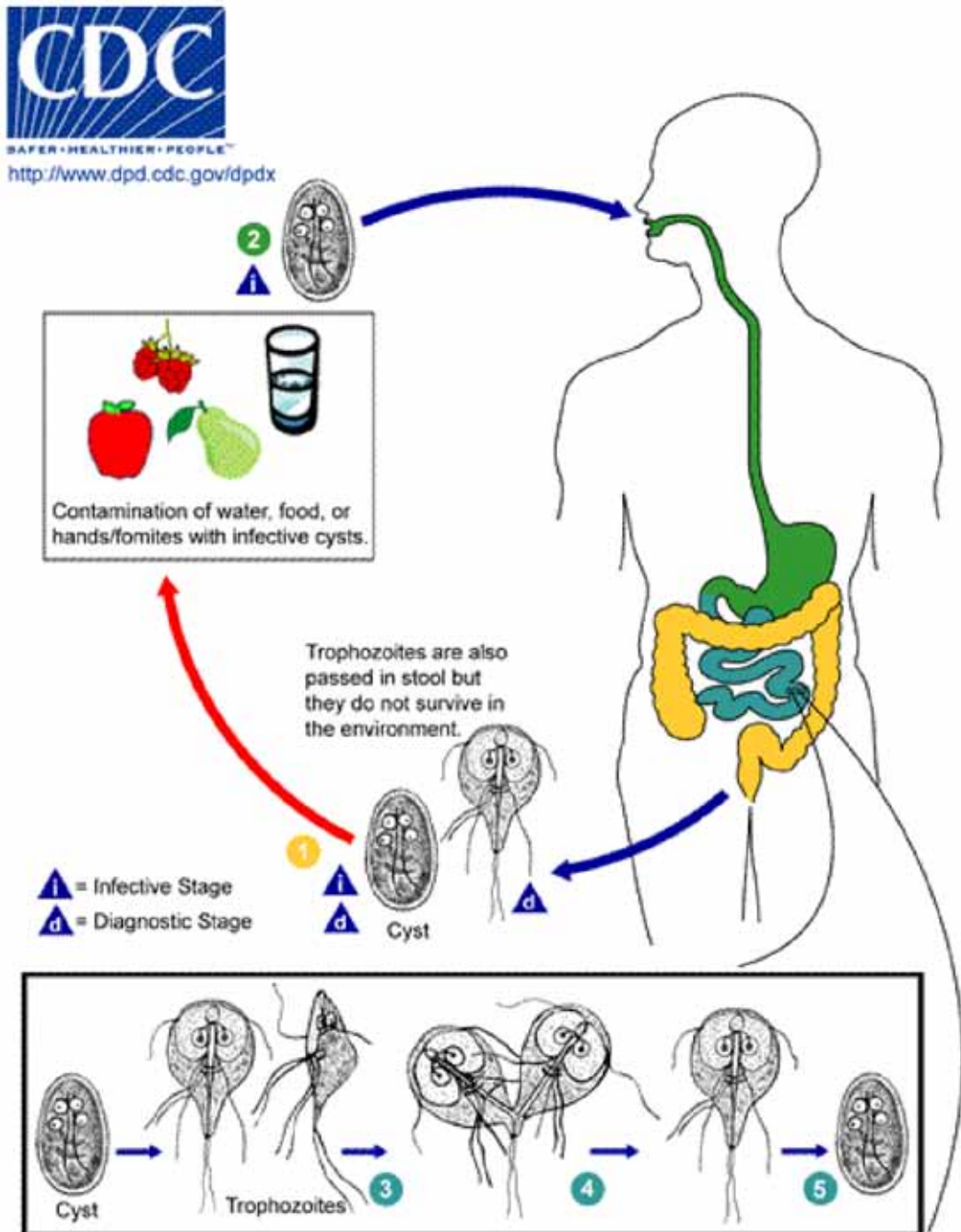


Figure 2.6. Life cycle of *Giardia duodenalis*
(Accessed from www.dpd.cdc.gov/dpdx)

body temperature and stable at a pH of 7.8 to 8.2. The parasite may also produce a lectin which, when activated by duodenal secretions, is able to facilitate attachment. Once attached, the organism is able to avoid peristalsis by trapping itself in between the villi or within the intestinal mucus.

Upon attachment to the intestinal cells, *G. duodenalis* is able to cause alterations in the villi such as villous flattening and crypt hypertrophy. These alterations lead to decreased electrolyte, glucose, and fluid absorption, and cause deficiencies in disaccharidases. Studies on *Giardia muris*-infected mice showed diffuse loss of microvillous surface area which investigators also correlated to decreased maltase and sucrase activities. The physiologic disturbances subsequently result in malabsorption and maldigestion, which in turn cause the signs and symptoms experienced by the patient. Bacterial colonization of the area may further worsen the damage already caused by the parasite.

In other studies, *G. duodenalis* was shown to rearrange the cytoskeleton in human colonic and duodenal monolayers. Cytoskeleton is essential for proper cell attachment to the extracellular matrix and the other neighboring cells. Changes observed in apoptotic cells include disruption of the cytoskeleton that leads to structural disintegration and detachment from the substrate. Hence, the parasite has been suggested to cause enterocyte apoptosis. This finding was strengthened by another study, which showed the ability of the parasite not only to disrupt cellular tight junctions but also to increase epithelial permeability, thus, leading to the loss of epithelial barrier function. With this loss of barrier function, luminal contents may penetrate the submucosal layers causing more damage in the intestinal tissue.

From ingestion of the cysts, it takes about 1 to 4 weeks (average of 9 days) for the disease to manifest. Half of the infected patients may

be asymptomatic. For acute cases, patients experience abdominal pain, described as cramping, associated with diarrhea. There is also excessive flatus with an odor of “rotten eggs” due to hydrogen sulfide. Other clinical features include abdominal bloating, nausea, and anorexia. Diarrhea is the most common symptom, occurring in 89% of cases. It is followed by malaise and flatulence. Spontaneous recovery occurs within 6 weeks in mild to moderate cases. In untreated cases, patients may experience diarrhea with varying intensities, for weeks or months.

Chronic infection is characterized by steatorrhea, or the passage of greasy, frothy stools. In some cases, periods of diarrhea have been observed to alternate with normal or even constipated bowel periods. There may be weight loss, profound malaise, and low-grade fever. In developing countries, it has been described as a cause of the failure-to-thrive syndrome.

Diagnosis

Diagnosis is made by demonstration of *G. duodenalis* trophozoites (Plate 2.11) and/or cysts (Plate 2.12) in stool specimens. Trophozoites in direct fecal smears may be characterized as having a floating leaf-like motility. To detect

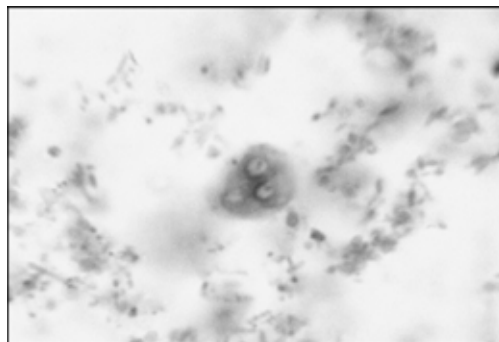


Plate 2.11. *Giardia duodenalis* trophozoite
(Courtesy of the Department of Parasitology,
UP-CPH)

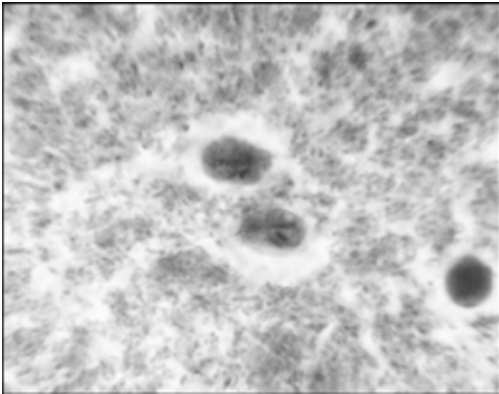


Plate 2.12. *Giardia duodenalis* cysts
(Courtesy of the Department of Parasitology,
UP-CPH)

cysts in stools, concentration techniques are recommended. At least three stool examinations on alternate days are recommended because of spotty shedding of cysts. If the parasite is not found in the feces, duodeno-jejunal aspiration may be done. Examination of the duodenal contents for trophozoites gives a higher percentage of positive findings compared to examination of feces. In a patient with chronic diarrhea, giardiasis should be considered as a possible cause.

Aside from duodenal aspiration, the Enterotest® (HDC Mountain View, CA) may demonstrate *Giardia* trophozoites. The patient swallows a gelatin capsule attached to a nylon string, with one end of the string attached to the patient's cheek. After about 4 to 6 hours, the string is removed, and any adherent fluid is placed on the slide for microscopic examination.

Presently, antigen detection tests and immunofluorescent tests are already available as commercial kits. Immunochromatographic assays detect the presence of *Giardia* antigen in stool. Cyst wall protein 1 (CWP1) is one of the antigens used for these diagnostic tests. Direct fluorescent antibody assays have been considered by many laboratories as the gold standard in diagnosis as such assays have

the highest combination of sensitivity and specificity.

Treatment

Giardiasis may be treated with metronidazole 250 mg three times a day for 5 to 7 days (pediatric dose: 15 mg/kg/day in three divided doses). Metronidazole is usually well-tolerated in adults and has a cure rate of 90%.

Alternative drugs include tinidazole (single dose of 2 g for adults; 50 mg/kg in children) and furazolidone (100 mg four times daily for 10 days for adults; 6 mg/kg/day in four divided doses for 7 to 10 days). Albendazole is an alternative at 400 mg/day for 5 days in adults and 10 mg/kg/day for 5 days in children. A meta-analysis has shown that albendazole is equally effective as metronidazole at the above doses. Although not available in the Philippines, nitazoxanide has likewise been used effectively in drug-resistant cases.

Prompt treatment of asymptomatic individuals reduce cyst passage and possible transmission especially among high risk groups such as food handlers, institutionalized patients, children attending day-care, and day-care workers.

Epidemiology

Giardia has a worldwide distribution. In the Philippines, the prevalence of giardiasis ranges from 1.6 to 22.0% depending on the population group being studied. From the local data, it can be clearly seen that the groups in areas with poor sanitation and hygiene practices have a higher prevalence of giardiasis.

Notably, *Giardia* is not commonly found in patients with diarrhea. In this symptomatic population, the prevalence was similar between children (<18 years old) and adults. However, the prevalence of giardiasis was significantly higher in male adults than females, a trend also seen in other countries (Table 2.2).

Table 2.2. Selected Philippine data on giardiasis

Reference	Population (n)	Prevalence
Cross and Basaca-Sevilla (1986)	Community (n=30,000)	6.0%
Auer (1990)	Urban poor, 8 months – 15 years (n=238)	20.0%
Bustos, et al. (1991)	Mentally ill patients (n=176)	17.0%
Lee, et al. (1999)	Children (Legazpi City) n=64	7.8%
Belizario, et al. (2000)	Patients from the community suspected with capillariasis (Brgy. Awao, Compostela Valley) (n=72)	4.2%
Belizario, et al. (2000)	Community (Brgy. San Isidro, Compostela Valley) (n=242)	7.4%
Avila, et al. (2003)	Food handlers, school canteen (n=123)	3.3%
Esparar, et al. (2003)	Food handlers, tertiary hospital (n=59)	3.4%
Kim, et al. (2003)	Community (n=301)	0.0%
Baldo, et al. (2004)	Institutionalized children (Metro Manila) (n=172)	11.6%
Belizario, et al. (2005)	Mall employees (Cebu city) (n=256)	0.8%
Natividad, et al. (2008)	Diarrheic patients (n=3,456)	2.0%
UP-CPH Department of Parasitology Laboratory (2006-2010)	Referred patients (n=667)	<1.0%
Yason and Rivera (2007)	Urban poor (n=2,354)	22.0%

The first published study on *Giardia* genotypes in the Philippines showed that the majority (86%) of the isolated genotypes belong to assemblage B.

Direct oral-anal sexual contact among men who have sex with men may increase the risk of giardiasis and infection with other intestinal protozoans.

Outbreaks of giardiasis are more frequently reported outside the Philippines. Most of these are water-borne (recreational water or drinking water). Foodborne outbreaks have also been reported. The low infective dose, prolonged communicability, and relative resistance to chlorine facilitate the transmission of *Giardia* through drinking and recreational water, food, and person-to-person contact.

Prevention and Control

Methods of prevention and control include proper or sanitary disposal of human excreta to prevent contamination of food and water supply. The former can be contaminated by the use of night soil as fertilizer, by flies,

or by infected food handlers. Normal water chlorination will not affect cysts, but usual water treatment modalities should be adequate.

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Trichomonas vaginalis

Juan Antonio A. Solon

Trichomonas vaginalis causes a sexually transmitted disease called trichomoniasis which has a worldwide distribution. Its incidence correlates strongly with the number of sexual partners. It was first observed by Donne in 1836 in purulent secretions of male and female urogenital tracts. It is now often described as the most prevalent non-viral sexually transmitted infection.

Parasite Biology

Trichomonas vaginalis exists only in the trophozoite stage. It has a pyriform shape, measuring 7 to 23 μm with four free anterior flagella that appear to arise from a simple stalk, and a fifth flagellum embedded in the undulating membrane. This membrane extends to about half the organism's length. The parasite has a median axostyle and a single nucleus.

The parasite is found in the urogenital tract. In women, it is found in the vagina but may ascend as far as the renal pelvis. The parasite can be isolated from the urethra, prostate, and less frequently, in the epididymis in men. The trophozoites multiply by binary fission in the host and are transferred passively from person to person (Figure 2.7). The usual mode of transmission is by sexual intercourse.

Pathogenesis and Clinical Manifestations

Inflammation of the vaginal mucosa occurs several days after the inoculation of *T. vaginalis* trophozoites. *T. vaginalis* cannot live without close association with the vaginal, urethral, or prostatic tissues. Four to 28 days after introduction of viable *T. vaginalis* into the vagina, proliferating colonies of the flagellate cause degeneration and desquamation of the vaginal epithelium followed by leukocytic inflammation

of the tissue layer. The trophozoites infect the surface but do not appear to invade the mucosa. The acute inflammation caused by the parasite results in the characteristic liquid vaginal secretions, greenish or yellow in color, that cover the mucosa down to the urethral orifice, vestibular glands, and clitoris. The vaginal secretions are very irritating and may cause intense itchiness and burning sensation. As the acute condition changes to the chronic stage, the secretion loses its purulent appearance due to a decrease in the trichomonads and leukocytes, an increase in epithelial cells, and the establishment of a mixed bacterial flora. Aside from the common symptoms of vaginal discharge, vulvitis, and dysuria, trichomonads appear to be associated with an increased incidence of postpartum endometritis. Complications in women include secondary bacterial infection of the urogenital tract.

Speculum examination reveals punctate hemorrhages of the cervix, the so-called strawberry cervix, which is observed in only 2% of cases.

Trichomonas infection in males may be latent and essentially asymptomatic. In some cases, it is responsible for an irritating persistent and recurring urethritis. Prostatitis is the most common complication.

Diagnosis

Saline preparation of vaginal fluid is the quickest and most inexpensive way to diagnose trichomoniasis, but the sensitivity of this technique is low at 60 to 70%. The accepted gold standard is culture which takes 2 to 5 days. The unstained wet drop preparations may be fixed and stained by Giemsa, Papanicolaou, Romanowsky, and

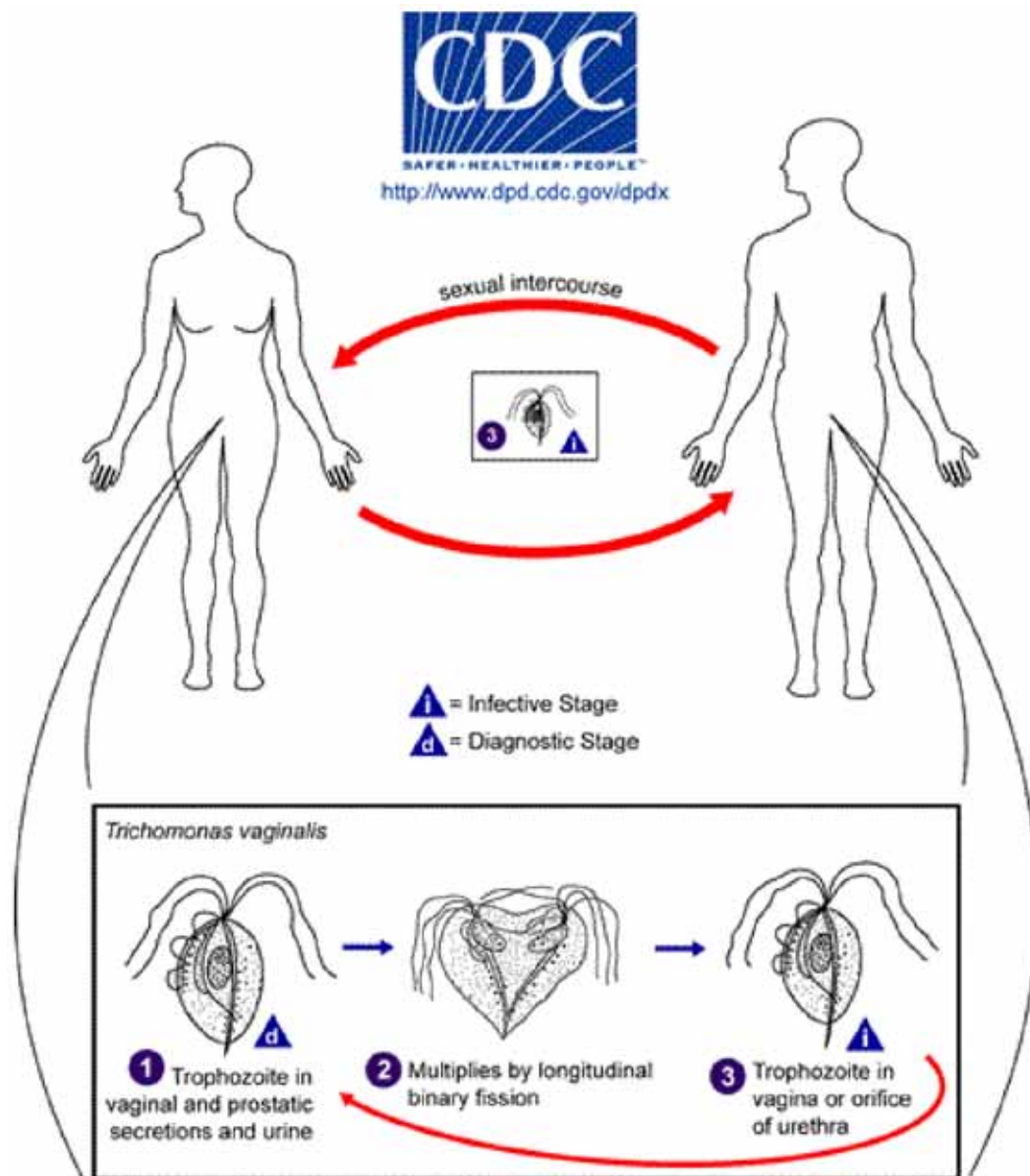


Figure 2.7. Life cycle of *Trichomonas vaginalis*
(Accessed from www.dpd.cdc.gov/dpdx)

acridine orange stains. *Trichomonas* can also be cultured using Diamond's modified medium, and Feinberg and Whittington culture medium.

The Pap smear may also show trichomonads (sensitivity 60%; specificity 95%). Antigen

detection tests and polymerase chain reaction (PCR) assays are commercially available, but not widely used locally. PCR among females does not seem to offer an added diagnostic advantage. Among males, however, diagnosis is

more difficult. For culture, the best results are seen with a combination of cultures of urethral swabs and urine sediment. PCR appears to detect more cases than culture among males.

The InPouch™ TV Test is a novel transport and culture test system which allows the specimen to be inoculated into a sealed pouch with culture media. Growth can be monitored microscopically directly through the pouch. This test has a comparable sensitivity to Diamond's modified medium culture.

Treatment

Trichomoniasis can be treated with metronidazole or tinidazole 2 g as a single dose. The reported cure rates of these drugs range from 86 to 100%. Sexual partners must be treated concomitantly to prevent reinfection. If metronidazole treatment failure occurs and reinfection is ruled out, a seven-day regimen of 500 mg metronidazole three times a day may be considered. If either this regimen fails, a 2 g daily dose for 5 days of either metronidazole or tinidazole can be used. In pregnancy, metronidazole remains the drug of choice for trichomoniasis.

Epidemiology

Trichomonas infection occurs worldwide. It is estimated that there are 170 to 190 million individuals with trichomoniasis. Prevalence is higher among women of child-bearing age. About 5 to 20% of women and 2 to 12% of men in developed countries are infected. Higher prevalence is associated with greater frequency of sexual intercourse with multiple partners and with commercial sex workers. Trichomoniasis is often associated with other sexually transmitted infections. In a study in the United States, 70% of male partners of women with trichomoniasis were likewise infected and the majority of the infected male partners were asymptomatic (77%).

In the Philippines, the prevalence of trichomoniasis among commercial sex workers varies with the method of diagnosis used, from 15% in studies using only microscopic examination of vaginal swabs to 37% in studies using culture. One study surveyed 421 male sex workers and there were no positive cases among them based on microscopy (Table 2.3). Local isolates of *T. vaginalis* have been

Table 2.3. Selected Philippine studies on trichomoniasis

Reference	Population (n)	Method	Prevalence
Arambulo, et al. (1977)	Waitresses/hostesses and housewives (n=560)	Microscopy	Among waitresses/hostesses: 15.0% Housewives: 2.7% Overall: 5.9%
Basaca-Sevilla, et al. (1986)	Total=1371 commercial sex workers (n=1,284) expectant mothers (n=87)	Microscopy and culture	24.0% on initial examination; 37.0% after 5 days of culture
Jueco, et al. (1998)	n=368 (women) 150 women from a private gynecologic clinic (housewives, workers, students, vendors, factory workers, business women, beautician) 218 commercial sex workers from a social hygiene clinic	Microscopy	Overall prevalence: 12.0% 8.0% from private clinic Among sex workers: 14.8%
Monzon, et al. (1991)	Total=1,357 commercial sex workers; females (n=936) males (n=421)	Microscopy	Females: 3.8% Males: 0.0%

characterized molecularly showing low genetic polymorphism.

It is relevant to discuss trichomoniasis in the context of HIV. In Zimbabwe and South Africa, trial participants diagnosed with trichomoniasis were more likely to test positive for HIV in their next visit. Perinatal transmission of HIV was likewise more likely if the mother had vaginal infections.

Prevention and Control

Prevention is best achieved by reducing the risk of exposure. Limiting the number of sexual partners, and proper use of protective devices such as condoms and spermicidal foams may help prevent infection. To prevent “ping-pong” or recurrent infections, there should be simultaneous treatment of sexual partners. Prompt follow-up of patients and their contacts, as well as health and sex education about venereal disease are also important.

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Non-Pathogenic Flagellates

Juan Antonio A. Solon

Trichomonas hominis

As with other *Trichomonas* species, *T. hominis* occurs only as a trophozoite which has a pyriform shape and measures 7 to 13 μm . It has five anterior flagella and a posterior flagellum projecting from an undulating membrane. The cytostome and the nucleus are situated at the anterior end. An axostyle extends from anterior to posterior along the mid-axis. Transmission occurs rapidly through fecal contamination of food and drinks.

Its habitat is the cecal area of the large intestine of human and other primates. It is non-invasive. Trophozoites pass out with diarrheic stools. The prevalence in the Philippines is less than 1%.

Trichomonas tenax

Trichomonas tenax is a pyriform flagellate which has been observed only in the trophozoite stage. It measures 5 to 12 μm , and is smaller and more slender than *T. vaginalis*. It has four free equal flagella and a fifth one on the margin of an undulating membrane which does not reach the posterior end of the body, and lacks a free posterior extension. It has a single nucleus and a cytostome. The organism multiplies by binary fission and thrives on the microorganisms found in its environment.

Exposure results from droplet spray from the mouth, kissing, or common use of contaminated dishes and drinking glasses. *Trichomonas tenax* is a harmless commensal of the human mouth, living in the tartar around the teeth, in cavities of carious teeth, and in necrotic mucosal cells in the gingival margins. It is quite resistant to changes in temperature and will survive for several hours in drinking water.

Diagnosis is made by swabbing the tartar between the teeth, the gingival margin, or tonsillar crypts.

Pulmonary trichomoniasis has been reported among those with underlying chronic pulmonary disease, entering the lungs most probably by aspiration. The parasite is probably unable to cause disease on its own. The presence of bacteria most probably allows it to proliferate profusely. In most of these cases, treatment with metronidazole results in rapid improvement.

Chilomastix mesnili

This organism inhabits the cecal region of the large intestine. It has well-defined trophic and cystic stages. The trophozoite is asymmetrically pear-shaped as a result of a spiral groove extending through the middle half of the body. Its size ranges from 6 to 10 μm . The characteristic boring and spiral forward movement is made possible by the three anterior free flagella and a more delicate one within the prominent cytostome.

The cyst is pear- or lemon-shaped, broadly-rounded at one end and somewhat bluntly-conical at the other end which has a knob-like protruberance that is visible occasionally. Internally, hematoxylin and eosin stained films clearly demonstrate the single large vestibular nucleus and the cytostome, which is almost as long as the encysted organism. Good preparations reveal a fibril on either side of the cytostome.

Transmission occurs through ingestion of cysts in food and drinks. Prevalence in the Philippines is less than 1%. This is a harmless commensal diagnosed by microscopic examination of feces and demonstration of either trophozoites or cysts. No treatment is

indicated. Preventive and control measures include improved sanitation and personal hygiene.

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Coccidians

Winifreda U. de Leon

The coccidian parasites are the largest group of apicomplexan protozoa falling under Class Conoidasida. Coccidia is a subclass of microscopic, spore-forming, single-celled obligate intracellular protozoan. Members of Phylum Apicomplexa are provided with a cluster of secretory organelles made up of rhoptries, micronemes, and polar rings with microtubules. In some species, a conoid may be found within the polar rings as well. The secretion allows the parasite to enter the host cell.

Coccidians infect the intestinal tract of most phyla of invertebrates and all classes of vertebrates including humans. They fall under Order Eucoccidiorida Suborder Eimeriorina. The disease called coccidiosis is recognized as one of the major problems in animal farming and in zoo management. Among humans, they are considered to be opportunistic in immunocompromised and immunodeficient individuals. Species with medical and veterinary significance include *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, *Sarcocystis*, and *Toxoplasma*.

In the coccidian life cycle, there is an alternation of sexual and asexual multiplication. It is typically characterized by three sequential stages, namely: sexual cycle or sporogony producing oocysts, asexual cycle or schizogony (merogony) producing merozoites (meronts), and gametogony resulting in the development of male (micro) and female (macro) gametocytes (gamonts). The complexity in the life cycles of coccidians is a challenge in terms of taxonomy.

Cryptosporidium hominis

There are several species of *Cryptosporidium* that are currently recognized. It was initially

reported that the only species that infect mammals was *C. parvum* and was believed to be the species infecting humans. However, molecular tools, especially DNA analysis, described the existence of another species, *Cryptosporidium hominis* found mainly in humans.

Parasite Biology

All stages of development are completed in the gastrointestinal tract of the host. Oocysts when passed out are already infective. Oocysts produced by *C. hominis* are found in the feces of humans and other animals. The oocysts are round and measure 4 to 5 μm in diameter. Each oocyst contains four sporozoites, which are present at the time of passage into the feces. The oocyst is infectious and when ingested, the sporozoites attach to the surface of epithelial cells of the gastrointestinal tract. The sporozoites develop into small trophozoites and become intracellular but extracytoplasmic, and attach to the brush borders. The trophozoites divide by schizogony producing merozoites that infect other cells. Macro- and microgametocytes are eventually produced, and the macrogamete is fertilized by the microgamete to produce a zygote. There are two types of oocysts resulting from the zygote: the thin-walled and the thick-walled oocysts. The thin-walled oocysts infect other enterocytes thus resulting in autoinfection, which is possibly responsible for the chronicity of the infection among the immunocompromised. On the other hand, the thick-walled oocysts are passed out with the feces that may contaminate food and water, which are ingested by the same or another host (Figure 2.8).

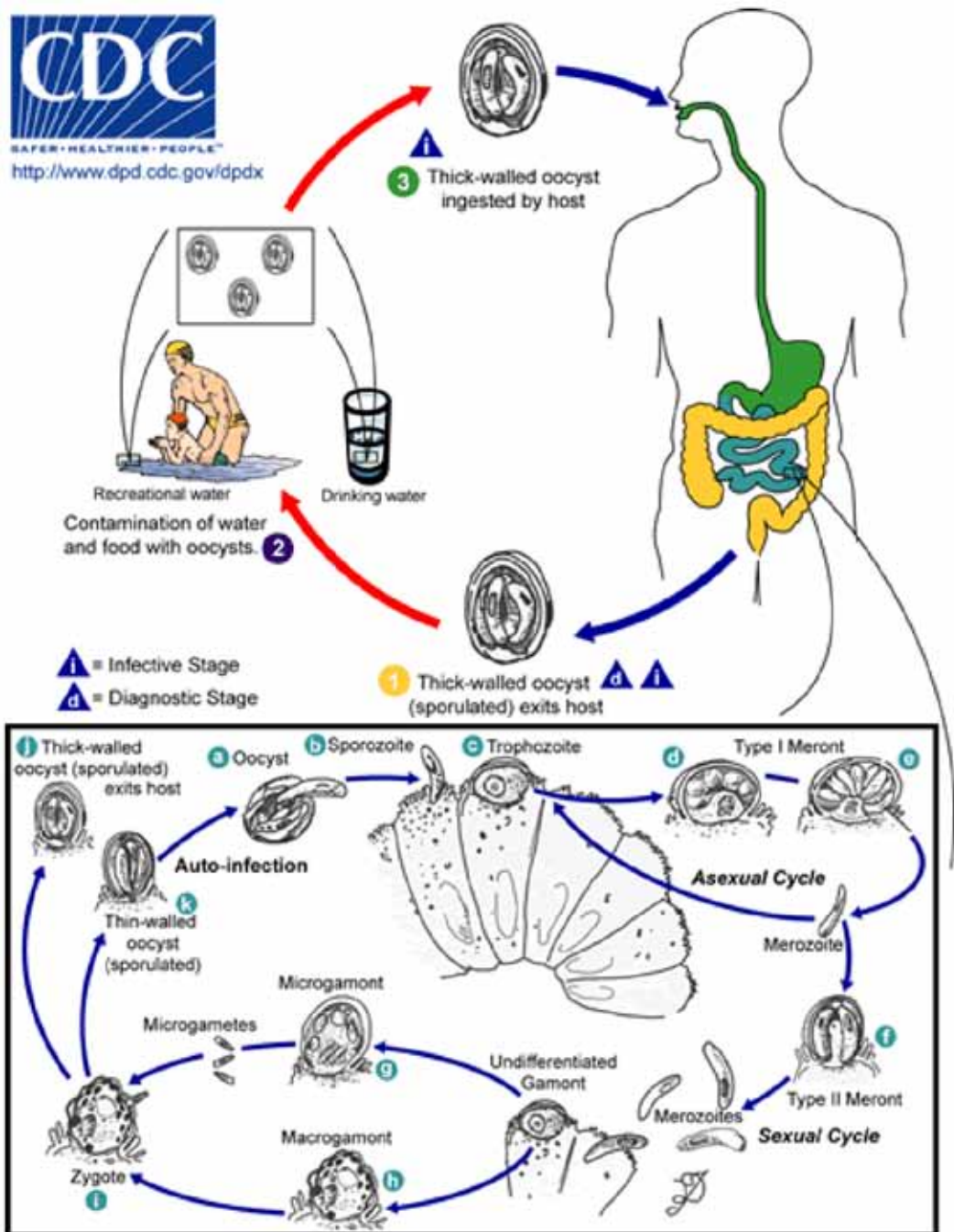


Figure 2.8. Life cycle of *Cryptosporidium* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

Pathogenesis and Clinical Manifestations

Cryptosporidiosis hominis was not well recognized prior to the occurrence of acquired immune deficiency syndrome (AIDS). In the immunocompetent host, the disease may present as a self-limiting diarrhea lasting for 2 to 3 weeks, and less commonly, abdominal pain, anorexia, fever, nausea, and weight loss. In immunocompromised persons, the diarrhea becomes more severe, progressive, and may become life-threatening. The bile duct and gall bladder may become heavily infected and lead to acute and gangrenous cholecystitis. Respiratory infections lead to chronic coughing, dyspnea, bronchiolitis, and pneumonia.

The villi of the intestines become blunted and there is infiltration of inflammatory cells into the lamina propria and elongated crypts. There may be varying degrees of malabsorption and excessive fluid loss in immunocompromised patients. Death may occur in disseminated infections.

Diagnosis

There are several methods of stool examination that will reveal *C. hominis* oocyst. Sheather's sugar flotation and the formalin ether/ethyl acetate concentration technique are commonly used. Kinyoun's modified acid-fast stain is routinely used with the oocysts appearing as red-pink doughnut-shaped circular organisms in a blue background. Intestinal biopsy material may also be examined under a light microscope and stages of the parasite can be seen at the microvillus region of the infected enterocyte. In cases of pulmonary involvement, the parasite may be recovered from the sputum, although transbronchial and broncho-alveolar lavage can yield a better result.

Indirect fluorescent antibody, enzyme immunoassay, and DNA probes specific for *C. hominis* have been developed. Acid-fast staining is probably the quickest and cheapest method of diagnosis.

Treatment

There is presently no acceptable treatment for cryptosporidiosis. Nitazoxanide, however, has been reported effective in preliminary trials. Bovine colostrum as well as paromomycin and clarithromycin have shown promise in treating severe diarrhea. Azithromycin may also be of value. In addition to chemotherapy, body fluid replacement and symptomatic treatment are recommended for both the immunocompetent and immunosuppressed patients.

Epidemiology

Cryptosporidiosis hominis has a universal distribution with infections reported worldwide. Epidemics are unusual in North America, although there was a report of an epidemic involving over 400,000 cases in the state of Wisconsin in the United States. This epidemic was attributed to the use of a faulty water purification system. Most epidemics are associated with water, and in many cases, the water was contaminated with calf feces. *Cryptosporidium parvum* of calves has been reported to cause infection among veterinary attendants and visitors in dairy farms and petting zoos.

Swimming in contaminated recreation water may result in accidental ingestion of infective oocysts. Swimming pool disinfection with 3 to 5 mg/L of chlorine does not kill the oocysts. The most common mode of transmission is from one person to another. Infected food handlers may likewise transmit oocysts during handling of beverages, raw vegetables, and other food that may be eaten raw. Unpasteurized milk, freshly pressed apple cider, potato salad, and sausages were found sources of infection. Nosocomial infections have also been reported among health workers caring for AIDS patients.

In developing countries, prevalence ranged from 3 to 20%. The prevalence in the Philippines has been reported to be low at 2.6%.

A study done in San Lazaro Hospital attempted to describe *Cryptosporidium* among diarrheic patients and reported a prevalence of 8.5%, while a study done in the Philippine General Hospital on diarrheic patients had a much lower prevalence at 1.7%.

Prevention and Control

Water-borne transmission is the most common source of cryptosporidiosis. Chlorination does not affect the parasite. The synergistic effect of multiple disinfectants and combined water treatment processes may reduce *C. hominis* oocysts in drinking water. Natural water and swimming pool water should not be swallowed. Contamination of drinking water by human and animal feces should be prevented.

Cyclospora cayetanensis

When first associated with diarrhea, this organism was thought to be a member of cyanobacteria because it showed photosynthesizing organelles and autofluorescing particles characteristic of the blue green algae.

Parasite Biology

Cyclospora cayetanensis was originally called a cyanobacterium-like body (CLB) but upon careful study, it was found to be a coccidian parasite. Similar to the other intestinal coccidians, the life cycle begins with the ingestion of sporulated oocyst, which contains two sporocysts with two sporozoites each. The released sporozoites invade the epithelial cells of the small intestines, although the site of predilection was found to be the jejunum. Multiple fissions of these sporozoites take place inside the cells to produce meronts, which contain 8 to 12 merozoites during the first generation, and only four merozoites in the second generation. Some of the merozoites develop into male (micro) and female (macro)

gametes. The microgametes fertilize the macrogametes to produce oocysts, which are passed out with feces when the host cells are sloughed off from the intestinal wall. The oocysts undergo complete sporulation within 7 to 12 days in a warm environment.

It is assumed that the oocyst is the infective stage and when ingested, the sporozoites are released and enter intestinal cells to go through schizogony and gametogony. The different developmental stages of the parasite may be found in the intestinal tissue (Figure 2.9).

Pathogenesis and Clinical Manifestations

Initial symptoms include malaise and low grade fever, which may occur 12 to 24 hours after exposure. Chronic and intermittent watery diarrhea occurs early in the infection and may alternate with constipation. The diarrhea may continue for 6 to 7 weeks with six or more stools per day. Other symptoms such as fatigue, anorexia, weight loss, nausea, vomiting, abdominal pain, flatulence, bloating, and dyspnea may develop. D-xylose malabsorption has been found to develop in some of the patients. Infections are usually self-limiting and immunity may result with repeated infections. No death has been associated with cyclosporidiosis.

Diagnosis

Direct microscopic examination of fecal smears under high magnification (400x) is recommended. Various concentration techniques and acid-fast staining (Kinyoun's stain) are also useful. Oocysts are auto-fluorescent and under fluorescent microscopy, they appear as blue or green circles depending on the filter (365-450 DM). This technique is useful for screening. Safranin staining and microwave heating are also helpful. A polymerase chain reaction (PCR) technique has been developed to differentiate *Cyclospora* from closely related *Eimeria* species.

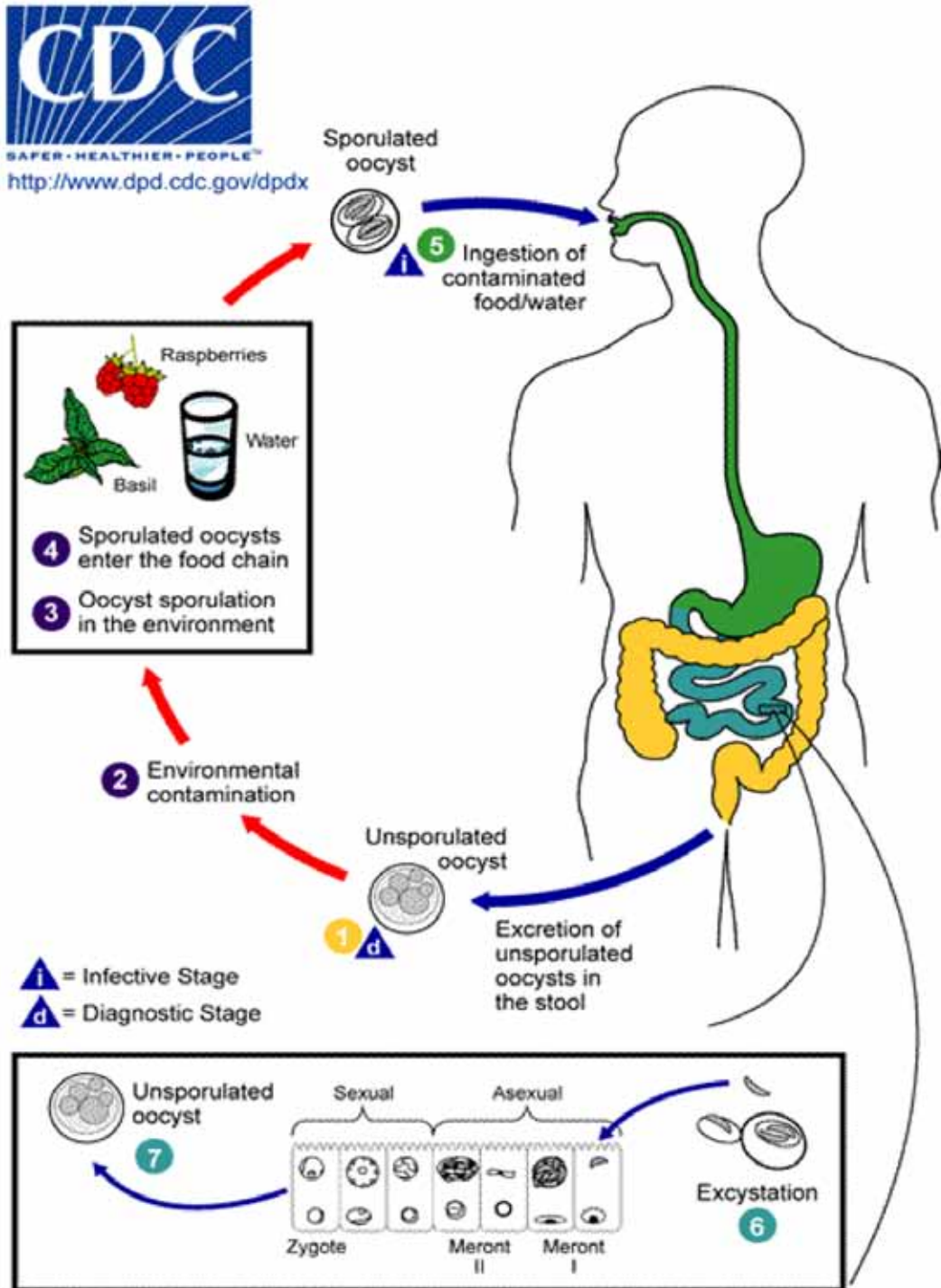


Figure 2.9. Life cycle of *Cyclospora cayetanensis*
(Accessed from www.dpd.cdc.gov/dpdx)

Treatment

The disease is self-limiting and treatment is not necessary if the symptoms are mild. If pharmacologic treatment is warranted, the only effective drug is trimethoprim-sulfamethoxazole 160/800 mg twice daily for 7 days. There is no alternate treatment if patients are unable to tolerate sulfamethoxazole. Oocysts disappear from the stools a few days after treatment. However, recurrence of symptoms was noted in about 40% of patients within 1 to 3 months post treatment.

Epidemiology

Cyclosporiasis has been described in many countries, with epidemics reported in Nepal, Peru, Haiti, and the United States. Infections were reported to appear in Nepal in late May and June and continued until October to November, the rainy season. Most cases in Nepal were reported in expatriates and tourists, and more recently in Nepali children and adults. In Peru, infections are commonly reported in children, while in Haiti, infections affect more homosexual males. Epidemics involving over 1,000 persons were reported in the United States in 1996 and 1997. Raspberries imported from Guatemala were incriminated in the infections in the United States. Leafy vegetables have been found to contain oocysts in Peru and Nepal, while lettuce and basil-pesto salad has been incriminated in other cases in the United States. Contaminated water is thought to be the main source of infection. No animal reservoirs have been found and, therefore, cyclosporiasis is presently considered mainly as a human disease. In the Philippines, a study of diarrheic stools from children in 2005 at the College of Public Health, University of the Philippines Manila, revealed a prevalence of 3.1% using safranin staining heated in a microwave.

Prevention and Control

Since the direct source of *C. cayetanensis* is unknown, good sanitary practices should be

followed to prevent the infection. Only water that has been subjected to adequate treatment procedures should be consumed. In most endemic areas, boiling water seems to be the best method since chlorination is not effective. Fruits and vegetables should be washed with clean water, but it would be prudent to avoid eating fruits and vegetables that have been exposed to natural untreated water. In Guatemala, it was believed that raspberries were exposed to oocysts in places where creek water was used to dilute insecticides sprayed on the plants. Similarly, in Nepal it is believed that cabbage became contaminated when watered with raw irrigation water.

Cystoisospora belli

This is the causative agent of a medical condition affecting the small bowel called cystoisosporiasis. The other known species *Isoospora hominis* is now taxonomically grouped under the genus *Sarcocystis*.

Parasite Biology

The sporulated oocyst contains two sporocysts each containing four sporozoites (infective stage). When ingested via contaminated water or food, the sporozoites excyst in the small intestine releasing sporozoites, which penetrate the epithelial cells, thus starting the asexual stage or the schizogonic phase of the life cycle (Figure 2.10). The sporozoites develop in the epithelial cell to form a schizont, which ruptures the host epithelial cell liberating merozoites into the lumen. These merozoites will then infect new epithelial cells and the process of asexual reproduction in the intestine continues. This process may continue for weeks or months. Some of the merozoites undergo gametogony to produce macrogametes and microgametes (sexual stages), which fuse to form a zygote that eventually matures to form an unsporulated oocyst. Sporulation usually occurs within 48 hours after passage with the stool.

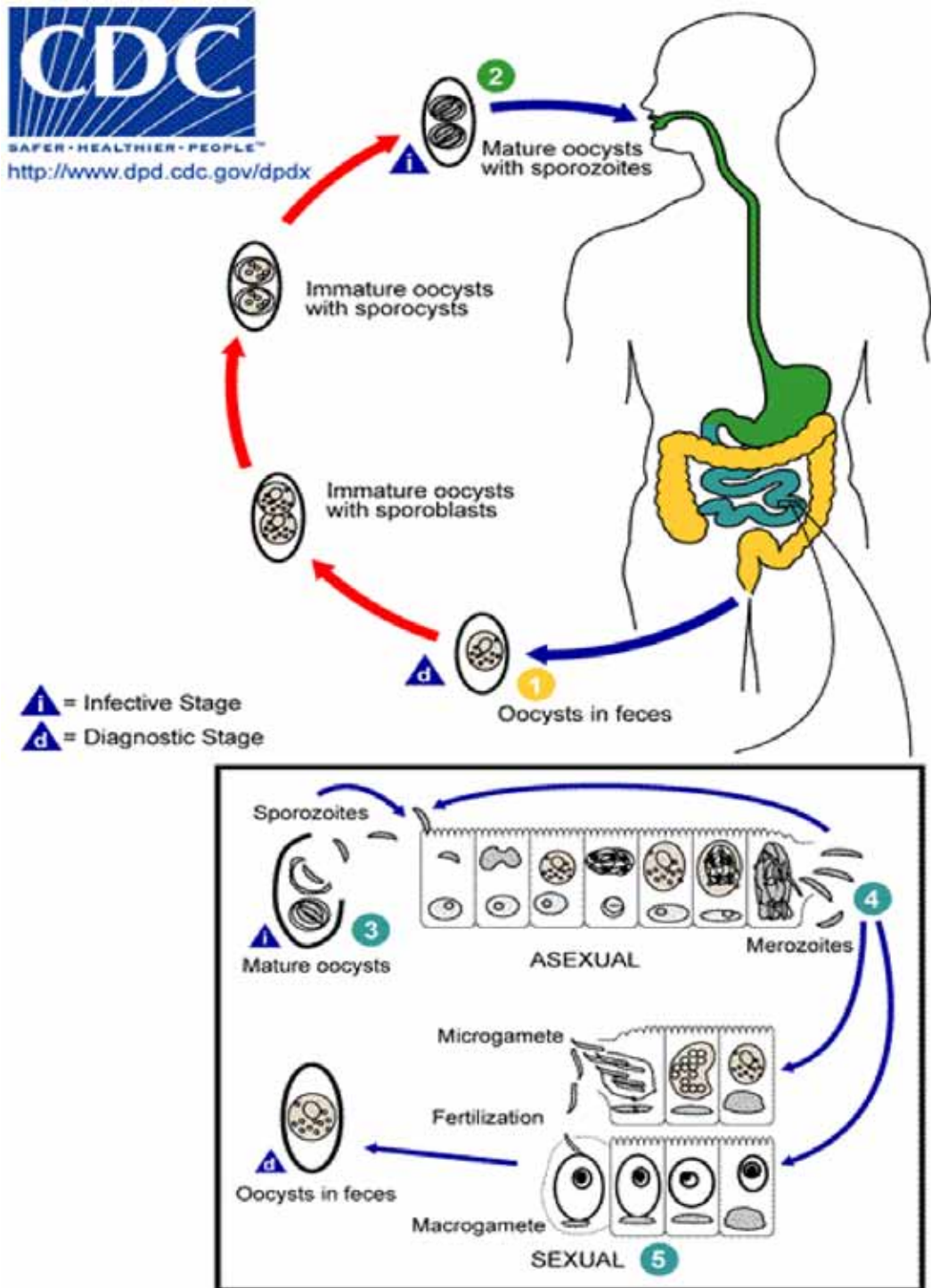


Figure 2.10. Life cycle of *Cystoisospora belli*
(Accessed from www.dpd.cdc.gov/dpdx)

Pathogenesis and Clinical Manifestations

Among the immunocompetent, infection is generally asymptomatic or may present as a self-limiting gastroenteritis. However, in more severe infections, severe diarrhea and fat malabsorption can occur. Symptoms include low-grade fever, anorexia, vomiting, general body malaise, anorexia, weight loss, and flatulence. Stools usually contain undigested food, mucus, and Charcot-Leyden crystals.

Infection in immunocompromised individuals ranges from a self-limiting enteritis to severe diarrheal illness resembling that of cryptosporidiosis, giardiasis or cyclosporiasis. Mucosal bowel biopsy may reveal flattened mucosa and damaged villi. Infiltration of the lamina propria with lymphocytes, plasma cells, and eosinophils has been reported. However, the mechanism by which the parasite produces these lesions is still not clear.

Diagnosis

The oocysts of *C. belli* may be detected in the feces by direct microscopy or formalin-ether/ethyl acetate concentration (Plate 2.13).

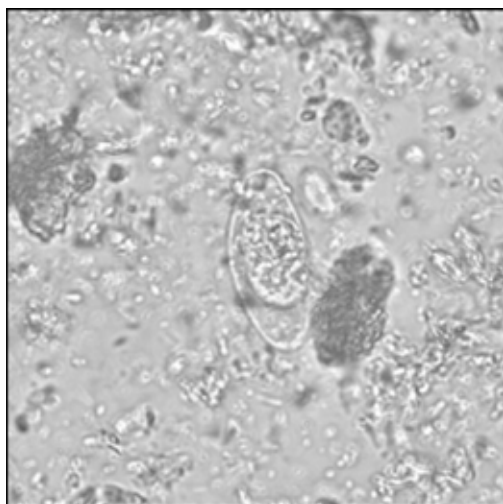


Plate 2.13. Immature oocyst of *Cystoisospora belli* recovered from stool sample, showing a single sporoblast
(Accessed from www.dpd.cdc.gov/dpdx)

Other concentration techniques that can also be used include zinc sulfate and sugar flotation. Oocysts are thin walled, transparent, and ovoid in shape. They appear as translucent, oval structures measuring 20 to 33 μm by 10 to 19 μm . Alternatively, oocysts can be seen in a fecal smear stained by a modified Ziehl-Neelsen method, where they stain granular red color against a green background. Phenol-auramine, as well as iodine staining of the specimen can help visualize the organism. Acid-fast stain, such as Kinyoun's stain or an auramine-rhodamine stain, is also useful. A considerable amount of stool may have to be examined because oocysts in the samples are often few in number. Charcot-Leyden crystals may be seen in the stool specimen. In blood examination, peripheral eosinophilia is common. String capsule (Enterotest®) and duodenal aspirate examinations may be of value. Molecular based techniques may prove useful as an additional diagnostic tool.

Treatment

Asymptomatic infections may be managed with bed rest and a bland diet, while symptomatic infections, such as those occurring in AIDS patients, can be treated with trimethoprim-sulfamethoxazole 160/800 mg four times per day for 10 days, then two times per day for 3 weeks. Combination therapy with pyrimethamine and sulfadiazine for 7 weeks has also been used successfully.

Epidemiology

Unlike the other coccidians, humans are the only known hosts of *C. belli*, which has a worldwide distribution. It is however more common in tropical and subtropical countries with poor sanitary conditions. The actual incidence of cystoisosporiasis is not known but *C. belli* has been tagged as the causative agent of diarrheal episodes in day care centers and mental institutions. The disease is common among patients with AIDS. In Africa, 2 to 3%

of those with AIDS were infected; in South America, 10%, and in Haiti and Africa, a range of 7 to 20% was observed. The disease has also been reported among those with lymphoma, leukemia, and organ transplants. Considered endemic are the following: Africa, Australia, the Caribbean Islands, Latin America, and Southeast Asia. Cystoisosporiasis has been reported in both adults and children, but severe diarrhea is common among infants. Both sexes were found susceptible to infection.

Prevention and Control

Cystoisosporiasis can be prevented by following good sanitary practices, thorough washing and cooking food, and drinking safe water.

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Toxoplasma gondii

Toxoplasma gondii is a coccidian that belongs to the Phylum Apicomplexa. It is a parasite that has a worldwide distribution and that infects humans and many species of animals.

Parasite Biology

The infective stages include the tachyzoite, the bradyzoite, and the oocyst. The complete life cycle occurs only in the members of the cat family (Felidae), which serve as definitive hosts. It follows a typical coccidian life cycle consisting of schizogony, gametogony, and sporogony in the intestinal epithelium. The extraintestinal stages are the asexual stages: tachyzoites and bradyzoites.

In the intestinal epithelium of the cat, merozoites multiply (schizogony) and then differentiate into microgametocytes and macrogametocytes (gametogony). Fertilization of the macrogamete by the microgamete gives rise to an oocyst. The oocyst is ovoidal in shape, has a thin wall, and measures 10 to 13 μm by 9 to 11 μm .

These oocysts are passed out with the feces of the cat in the unsporulated stage. These can be ingested together with contaminated food or water by another host. The oocysts complete sporulation within three to four days. Inside the mature oocyst, two sporocysts are formed, each having four sporozoites. When the mature oocyst reaches the intestine of the new host, it excysts and releases four sporozoites which can penetrate the lamina propria. The parasites gain entry to the lymphatics then spread to the different organs, tissues, and fluids of the body (Figure 2.11).

Toxoplasma is an intracellular parasite, which infects different kinds of nucleated cells including macrophages. Following the entry of the sporozoite into a new cell, it transforms

into a tachyzoite (Plate 2.14). Tachyzoites are found during the initial and acute stage of the infection, but as host immunity to the parasite is developed, the fast multiplying tachyzoites give rise to slow multiplying bradyzoites that form cysts. Only these two stages are present in humans and other animal intermediate hosts. Asexual multiplication is by a variation of binary fission called endodyogeny. This is characterized by the formation of the plasma membrane by the two new daughter parasites, even before the division of the nucleus. Cells in which endodyogeny occur eventually burst, thus liberating trophozoites that invade other cells. It is possible that tachyzoites can be transferred from one person to another by granulocyte blood transfusion. Tachyzoites can be transferred from the newly infected mother to the fetus during the first trimester of pregnancy by passing through the placental barrier. Tachyzoites and bradyzoites can be transferred by organ transplant especially bone marrow, and bradyzoites can be acquired by eating meat of infected animals.

The trophozoite measures 4 to 8 μm in length, 2 to 3 μm width. It is crescent-shaped with a pointed anterior and a rounded posterior end. Organelles, such as rhoptries and micronemes, which are associated with cell penetration, are found in a short conoid on the anterior end. A spherical nucleus is found in the posterior end. In the infected macrophage, the parasites prevent the fusion of the parasitophorous vacuole that contains the parasites, with the lysosome and are, thus, not killed by the lysozyme. Pseudocysts containing proliferating tachyzoites are seen in tissue sections taken from patients suffering from acute infection. These do not have well-formed walls unlike cysts containing many bradyzoites

into bradyzoites that are protected by a cyst wall and proliferate at a slower rate. Cysts can be found in the brain, skeletal and heart muscles, and retina. Clinical manifestations become apparent when the immune system is suppressed as in old age, drug-induced immunosuppression after organ transplantation, or in the case of AIDS. More often, symptoms appear when there is relapse of chronic infections as a result of a suppressed immune system rather than as a response to an acute infection. Among the immunocompromised patients, the most common manifestation is encephalitis. Myocarditis and focal pneumonia have also been reported. It is also possible that the immunosuppressed patient acquires the infection from blood transfusion or organ transplantation. Clinical manifestations include retinochoroiditis, lymphoreticular hyperplasia with enlargement of the posterior cervical lymph node, hepatitis, splenomegaly, pneumonia, extramedullary hematopoiesis, and failure to gain weight.

Stillbirth and abortion may result when mothers acquire the infection during the first trimester of pregnancy. Babies may exhibit clinical manifestations like chorioretinitis, epileptic seizures, jaundice, hydrocephaly, and microcephaly. Death of the infected newborn babies is usually due to anemia with pneumonia. There are cases when clinical manifestations may not be apparent during the neonatal period, but will appear later in childhood. Most babies will harbor the infection and grow up without any clinical manifestation until such time later in life when their immune system is suppressed and there is reactivation of chronic toxoplasmosis.

Diagnosis

Identification of the parasite can be done through examination of tissue imprints stained with Giemsa. Tissue sections can be processed and stained with hematoxylin and eosin. Serodiagnostic methods are used to detect

antibodies against *T. gondii*. A seroconversion to a positive titer or a four-fold increase in titers is indicative of an infection. The Sabin-Feldman methylene blue dye test is very sensitive and specific but it requires the maintenance of live organisms in the laboratory. High titers (>1,024), although usually indicating an acute infection, may also be seen in chronic cases, hence the need for IgM antibody detection through either the IgM indirect fluorescent antibody technique or through a double sandwich IgM enzyme immunoassay. Handling of live trophozoites may result in accidental infection of the laboratory personnel. Other tests are the indirect hemagglutination test, indirect fluorescent antibody test, and enzyme-linked immunosorbent assay. Latex agglutination test is also available. Differentiating pre-existing antibody from passively transferred antibody from the mother or antibody related to illness is important in the assessment of serological test results.

Better diagnostic assays are being developed because toxoplasmosis has been recognized as an important disease associated with AIDS. Polymerase chain reaction (PCR) has been successfully used in the diagnosis of toxoplasmosis using samples taken from the patient, which include serum, amniotic fluid, cerebrospinal fluid, and bronchoalveolar lavage, especially in cases where there is very little amount of specimen available.

Treatment

Treatment consists of pyrimethamine (25-100 mg daily) and sulfadiazine (1-1.5 g four times daily) used in combination for one month. These drugs keep the *Toxoplasma* under control but do not kill it. Since pyrimethamine can lower blood counts in most people, it should be given together with leucovorin (folic acid). Sulfadiazine may cause serious allergic reactions like fever and rash, but it can be substituted with clindamycin. Spiramycin, azithromycin, clarithromycin, dapsons, and

atovaquone may also be used. Corticosteroids are sometimes given to prevent occurrence of hypersensitivity reactions. Prophylaxis with trimethoprim-sulfamethoxazole may be given for the immunocompromised.

Epidemiology

Toxoplasmosis is endemic worldwide in humans and in domestic and wild animals as well. Disease due to this parasitic infection is not manifested except in cases of immune deficiency or suppression. Determination of the prevalence of infection is based on serodiagnostic tests, although these tests are not readily available in the Philippines due perhaps to a lack of demand since clinical toxoplasmosis is not common. According to surveys by Cross and Basaca-Sevilla, only 2.4% of the population is seropositive for *Toxoplasma gondii*. Pigs and rats, however, have a higher prevalence of positive titers for *Toxoplasma* antibodies at 19% and 8.1%, respectively.

Prevention and Control

Food should be protected from contamination with cat feces. Meat and eggs should be well cooked. Unpasteurized milk should be avoided. Pregnant women should avoid contact with cats. Laboratory workers should be very careful in handling the parasite.

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Sarcocystis spp.

Alice Alma C. Bungay, Raezelle Nadine T. Ciro

Sarcocystis is a genus of intracellular protozoa reported to infect humans and animals worldwide. Infection with this parasite is known as sarcosporidiosis or sarcocystosis. Species belonging to this genus infect a wide variety of animals such as birds, reptiles, and mammals. While majority of the species infect mammals, about a dozen are known to infect snakes.

This parasite was first reported in 1843 by Miescher as white threadlike cysts in striated muscles of a house mouse. It was simply referred to as Miescher's tubules until 1899, when the name *Sarcocystis miescheriana* was proposed to identify the said parasite. Since its discovery, it has been debated whether *Sarcocystis* spp. were protozoa or fungi. The debate was resolved only in 1967 when bradyzoites in the sarcocysts were studied under the electron microscope and were seen to possess organelles found in other apicomplexan protozoa such as *Toxoplasma* and *Eimeria*.

There are about 130 recognized species under *Sarcocystis* including *S. hominis* and *S. suihominis*. Humans serve as definitive hosts for the two species, but occasionally, humans can act as intermediate hosts. There is an ongoing revision of the taxonomy of this genus, and it is possible that all the currently recognized species may be fewer or may in fact be a single species that can infect multiple hosts.

Parasite Biology

Sarcocystis can take several forms. The simplest form is called a zoite. It is a banana-shaped cell, with a pointed anterior end, also known as the apical complex, which possesses micronemes, micropores, and rhoptries, and believed to be associated with host cell penetration and creation of an intracellular

environment suitable for parasite growth and development.

Sporulated oocysts and individual sporocysts can be passed out in the feces of an infected definitive host. The sporulated oocyst undergoes sporogony creating two sporocysts. Once sporogony is complete, the oocyst itself undergoes lysis, releasing the sporocysts into the environment. Sporocysts of most species measure 15 to 19 μm by 8 to 10 μm , and contain four sporozoites and a discrete refractile residual body. Sporocysts are capable of surviving on the ground and infecting intermediate hosts (Figure 2.12).

After oocysts and/or sporocysts are ingested by a susceptible intermediate host (usually cows or pigs), the sporocysts pass to the small intestine. The plates forming the sporocyst wall separate, releasing the four sporozoites into the intermediate host's body. The sporozoites migrate through the gut epithelium and eventually enter the endothelial cells in small arteries where they undergo the first two generations of asexual reproduction (called schizogony or merogony). These cycles result in the development of meronts. This stage lasts about 15 to 16 days after ingestion of sporocysts. Merozoites emerge from the second generation meronts and enter the mononucleate cells where they develop. Subsequent generations of merozoites develop in the direction of blood flow to arterioles, capillaries, venules, and veins throughout the body. The third asexual generation appears as multinucleate schizonts in capillaries throughout the body. Merozoites from this generation form metrocytes and encyst in the muscles, initiating sarcocyst formation.

Sarcocysts begin as unicellular bodies containing a single metrocyte. Through

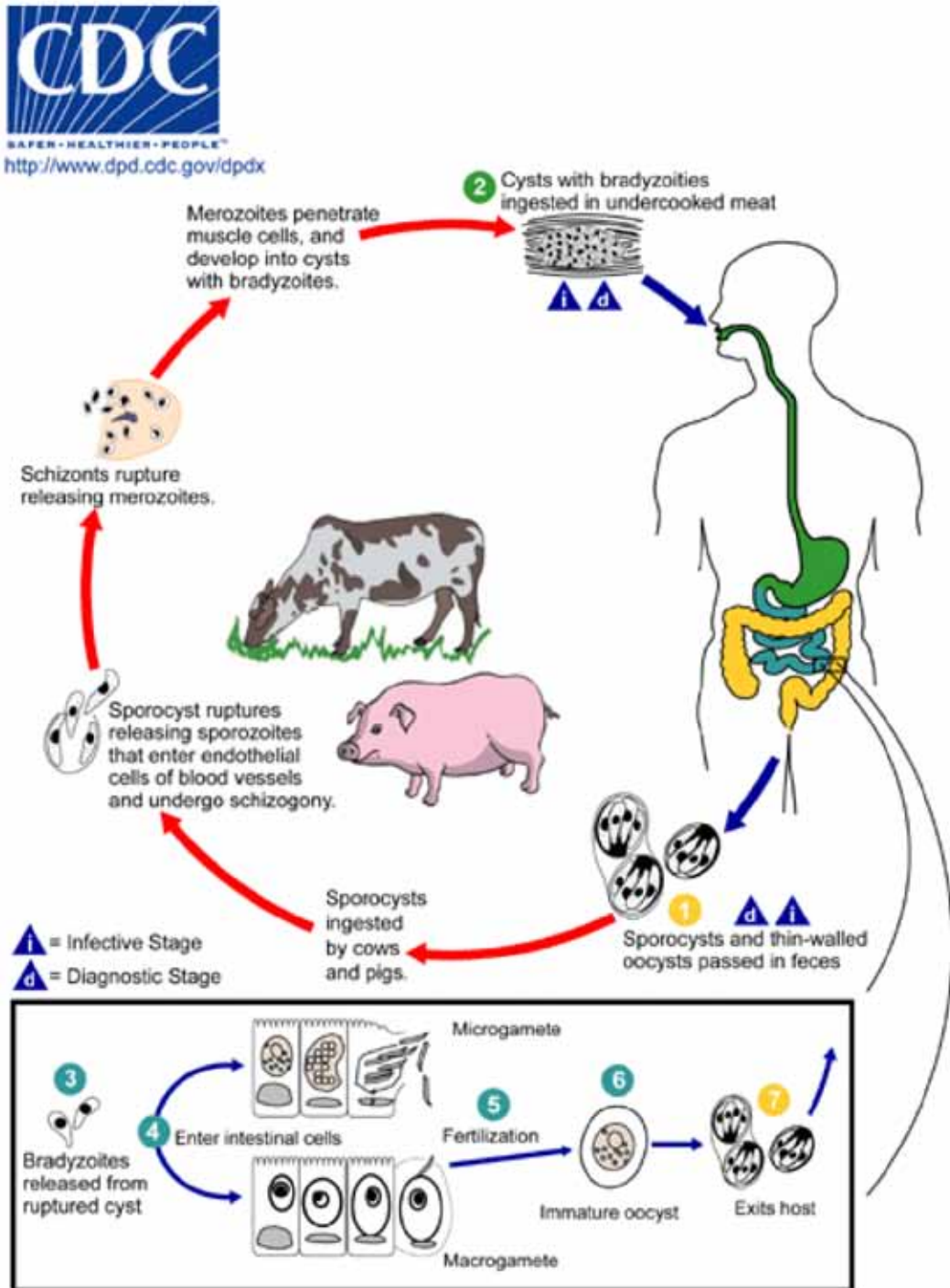


Figure 2.12. Life cycle of *Sarcocystis* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

repeated asexual multiplication, numerous metrocytes accumulate and the sarcocyst increases in size. As sarcocysts mature, the small, rounded, non-infectious metrocytes give rise to infectious, crescent-shaped bodies called bradyzoites. About two and a half months after infection, sarcocysts are already mature and are able to infect the definitive host.

Humans, as well as other definitive hosts, are infected by consumption of uncooked or undercooked meat of intermediate host that contains sarcocysts. Once the intermediate host is eaten by a definitive host such as dog or human, the parasite undergoes sexual reproduction within the intestines. After sarcocysts are ingested and the wall is digested, bradyzoites become motile. Active bradyzoites enter intestinal cells and change into the male and female forms, microgamonts and macrogamonts, respectively. Fusion of a macrogamont and a microgamont creates a fertilized cell called a zygote, which develops into an oocyst (containing two sporocysts). The oocyst is passed through the feces of the definitive host. Most definitive hosts do not show any clinical signs or symptoms.

More recently, a second life cycle has been described whereby carnivores and omnivores pass the infectious stages in their feces. Ingestion of this contaminated material may lead to successful infection.

Pathology and Clinical Manifestations

The pathology is of two types: a rare invasive form that presents with vasculitis and myositis, and an intestinal form that presents with nausea, abdominal pain, and diarrhea. While normally mild and lasting under 48 hours, the intestinal form may occasionally be severe or even life threatening. The invasive form may involve a wide variety of tissues including lymph nodes, muscles, and the larynx.

In studies where volunteers ingested infected beef, symptoms appeared 3 to 6 hours after eating. These symptoms included

anorexia, nausea, abdominal pain, distension, diarrhea, vomiting, dyspnea, and tachycardia. All symptoms were transient and lasted about 36 hours.

Sarcocystosis has also been associated with acute fever, myalgias, bronchospasm, pruritic rashes, lymphadenopathy, subcutaneous nodules with concurrent eosinophilia, elevated erythrocyte sedimentation rate, and elevated creatine kinase levels. Symptoms may last as long as 5 years. Segmental necrotizing enteritis has also been reported in one study.

Diagnosis

Presumptive diagnosis of human intestinal sarcocystosis is based on symptoms manifested by infected individuals and a history of recent consumption of raw or undercooked meat.

Identification of sporocysts in feces may require several stool examinations done on separate days during the infection. Sporocysts of *S. hominis* are first excreted 14 to 18 days after ingesting beef, and those of *S. suis* are excreted 11 to 13 days after ingesting pork. A fecal flotation wet mount is usually done to visualize sporocysts using bright-field microscopy. Flotation methods based on high-density solutions incorporating sodium chloride, cesium chloride, zinc sulfate, sucrose, Percoll, Ficoll-Hypaque, and other density gradient media are preferred over formalin-ether/ethyl acetate and other sedimentation methods. Species cannot be distinguished from one another solely by microscopy because sporocysts of different species overlap in size and shape.

Definitive diagnosis can be made through biopsy of an infected muscle. Sarcocysts of *S. hominis* are microscopic in muscles of cattle, whereas those of *S. suis* are macroscopic in muscles of swine. Sarcocysts are identifiable with hematoxylin and eosin stain. Confirmatory staining with the periodic acid-Schiff (PAS) can be performed as the walls stain positively. The walls of the sarcocyst may be used in species

diagnosis. Currently, 24 wall types have been identified in 62 species. *S. hominis* and *S. suihominis* both have walls of type 10. The wall of *S. hominis* is up to 6 µm thick and appears radially striated from villar protrusions that are up to 7 µm long. The wall of *S. suihominis* is 4 to 9 µm thick, with villar protrusions up to 13 µm long.

Recently, polymerase chain reaction (PCR) amplification of the 18S rRNA was demonstrated to be useful in distinguishing *S. hominis*, *S. fusiformis*, and *S. cruzi* sarcocysts and oocysts. The technique makes possible amplification and identification of species-specific gene sequences based on DNA extracted from as few as seven excreted sporocysts (the equivalent of 3 ½ oocysts) from freshly prepared material, or as few as 50 sporocysts from fecal samples that had been stored in potassium dichromate ($K_2Cr_2O_7$) for as long as 6 years.

Treatment

Because infection is often asymptomatic, treatment is rarely required. There have been no published trials so treatment remains empirical. Agents that have been used include albendazole, metronidazole, and co-trimoxazole for myositis. Corticosteroids have also been used for symptomatic relief.

Epidemiology

There are very few large-scale population surveys that have been conducted for *Sarcocystis* in humans. Prevalence data for *Sarcocystis* infections often come from case reports and findings of physicians, public health workers, and scientists with specific interests.

Human infection is considered rare with less than 100 published cases of invasive disease (approximately 46 cases reported by 1990). These figures may represent a gross underestimate of the human burden of disease. Sarcocystosis has been reported in Africa, Europe (Germany, Spain, and Poland), the United States (California), Central and South

America, China, India, Tibet, and Southeast Asia.

Of fecal specimens examined from children in Poland and Germany, 10.4% and 7.3% were found positive, respectively. In Tibet, *Sarcocystis* was detected in 42.9% of beef specimens examined from the marketplace, and *S. hominis* and *S. suihominis* were found in stool samples of 21.8% and 7% of 926 persons, respectively. Stool examination among Thai laborers showed that *Sarcocystis* infection had a prevalence of about 23%; all cases were asymptomatic which probably explained the lack of recognition. A study of 100 human tongues obtained post mortem in Malaysia revealed an infection rate of 21%. There was no sex difference and the age range was 16 to 57 years (mean 37.7 years). A seroepidemiological survey in West Malaysia found that 19.7% of 243 persons had antibodies for *Sarcocystis*.

In the Philippines, studies involving the examination of muscle tissues obtained from water buffaloes, cattle, pigs, and goats revealed the presence of *S. cruzi* in backyard cattle (*Bos taurus*) possessing a type 7 sarcocyst wall, *S. levinei* in water buffaloes (*Bubalus bubalis*) possessing a type 7 sarcocyst wall with similarities to *S. cruzi*, *S. miescheriana* in domestic pigs (*Sus scrofa domestica*) with a type 10 sarcocyst wall, and *S. capracanis* in domestic goats (*Capra hircus*) with a type 14 sarcocyst wall. There is a lack of local studies on human sarcocystosis.

Prevention and Control

Intestinal sarcocystosis can be prevented by thoroughly cooking or freezing meat to kill bradyzoites in the sarcocysts. Alternatively, freezing the meat at -5°C for several days will kill the sporocysts. Where contaminated drinking water is suspected, boiling should be considered to ensure disinfection.

The administration of anticoccidial drugs, amprolium and salinomycin, as chemoprophylactic agents was effective

in preventing severe illness and death in experimentally infected calves and lambs.

The risk of foodborne zoonoses warrants prevention and control in food animals. To avoid infection of food animals, they must be prevented from ingesting the sporocyst stage from human feces in contaminated water, feeds, and bedding. If such measures cannot be assured and meat is suspected to harbor cysts, the extent of infestation must be considered. In heavy and widespread infestations with visible cysts, the whole carcass must be condemned. In lighter infestations, those parts of the carcass which are not affected are passed for human consumption.

No vaccines are currently available. Experimentally inoculated pigs appear to develop a persistent immunity, hence, vaccine development may be explored.

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Other Intestinal Protozoans

Winifreda U. de Leon

Blastocystis hominis

Blastocystis hominis is an intestinal protozoan found in a vast array of animals, including humans. The classification of *Blastocystis* has been a long-standing problem for taxonomists. It was previously classified as yeast under the genus *Schizosaccharomyces*, while other taxonomists suggested that it was related to *Blastomyces* based on its glistening appearance in a wet mount and the absence of any organelle of locomotion.

Correlative light electron microscopy has since shown that the organism lacks a cell wall. It possesses nuclei, endoplasmic reticulum, Golgi complex, and mitochondrion-like organelles that are compatible with protozoan morphology. It is capable of pseudopodial extension and retraction. Moreover, the organism does not grow on fungal culture media. It responds to anti-protozoal drugs. Studies with cultured organisms have shown that reproduction is asexual, either through binary fission or sporulation under strict anaerobic conditions. Optimal growth is at 37°C in the presence of bacteria. All the above findings supported the reclassification of *B. hominis* from a yeast to an emerging human protozoan parasite.

There are new research findings on the taxonomic classification of *B. hominis*. In 1996, Silberman et al. completed a study of the small subunit rRNA (SSUrRNA) gene of the organism, and the results showed that it belongs to an informal group called Stramenophiles, which is a recently recognized group of microscopic parasites. This includes heterogenous protists like brown algae, diatoms, and water molds, to name a few.

Parasite biology

The life cycle is unclear. It has been proposed that the life cycle begins with ingestion of cysts from contaminated food or water. Upon ingestion, the cyst possibly develops into other forms, which may in turn re-develop into cyst forms. When excreted with stools, the cysts contaminate the environment and are eventually transmitted to humans and other animals through the fecal-oral route, repeating the cycle. Because the life cycle is not fully understood, validation of this proposed life cycle and the mode of transmission needs experimental confirmation. Multiplication of *B. hominis* is by binary fission.

B. hominis is known to occur in four morphological forms: (a) vacuolated, (b) ameba-like, (c) granular, and (d) multiple fission. More recently, additional cyst and avacuolar forms have been recognized.

Vacuolated forms are the most predominant forms in fecal specimens. These are spherical in shape, measuring 5 to 10 µm in diameter. A large central vacuole pushes the cytoplasm and the four nuclei to the periphery of the cell. Sometimes, a very thick capsule surrounds the vacuolated forms. The prominent central vacuole has been found to be a reproductive organelle. The vacuolar forms are considered to be the main type of *Blastocystis* that cause diarrhea.

Ameba-like forms, usually measuring between 2.5 to 8 µm, are occasionally observed in stool samples. They exhibit active extension and retraction of pseudopodia. The nuclear chromatin, when visible, characteristically shows peripheral clumping. The amebic form appears to be an intermediate stage between

the vacuolar form and the precystic form, as this stage allows the parasite to ingest bacteria in order to enhance encystment. Studies of Tan and Suresh have revealed that the ameboid forms predominated in isolates from symptomatic cases.

Granular forms are multinucleated and are mainly observed from old cultures. The diameter of the cell varies from 10 to 60 μm . The granular contents develop into daughter cells of the ameba-form when the cell ruptures.

Multiple fission forms arise from vacuolated forms. It is believed that these multiple fission forms produce many vacuolated forms.

The size of the resistant cystic form is about 3 to 10 μm in diameter, and has one or two nuclei. It has a very prominent and thick, osmophilic, electron dense wall. It appears as a sharply demarcated polymorphic, but mostly oval or circular, dense body surrounded by a loose outer membranous layer. This membranous layer seen in phase contrast microscopy corresponds to the fibrillar layer described around the cyst at the ultrastructural level, and is the easiest diagnostic feature to identify.

It is postulated that the thick-walled cyst may be responsible for external transmission, while those cysts with thin walls may be the cause of reinfection within a host's intestinal tract.

Pathogenesis and Clinical Manifestations

Infection with *B. hominis* is called blastocystosis. *B. hominis* as a cause of gastrointestinal pathology is controversial. Several studies have shown that the presence of the parasite in a majority of patients was not associated with symptoms; or, it was found with other organisms that were more likely to be the cause of the symptoms. However, other studies have concluded that the presence of *Blastocystis* in large numbers produces a wide variety of intestinal disorders, such as abdominal cramps, irritable bowel syndrome, bloating,

flatulence, mild to moderate diarrhea without fecal leukocytes or blood, nausea, vomiting, low grade fever, and malaise. Symptoms usually last about 3 to 10 days, but may sometimes persist for weeks or months.

It has been found that in subjects suffering from immunosuppression, *Blastocystis* showed a significant association with gastrointestinal symptoms. Other studies have also provided evidence of changes in the cellular immune function of infected individuals.

Diagnosis

Specific diagnosis based on clinical presentation alone may prove difficult, because the spectrum of symptoms is seen in other intestinal infections. Laboratory detection of the organism from stool is needed to confirm the diagnosis. Multiple stool samples should be collected from patients showing clinical signs and symptoms. Microscopic examination using direct fecal smear is useful, but sensitivity is increased when concentration techniques are used. Hematoxylin or trichrome staining offers a very convenient and easy method to differentiate the various stages of *Blastocystis*. Leukocytes are usually seen in fecal smears and stool eosinophilia may also be observed. The organism can be cultured using the Boeck and Drbohlav's or the Nelson and Jones media.

Treatment

Blastocystis is difficult to eradicate. It hides in the intestinal mucus, as well as sticks and holds on to intestinal membranes. The drug of choice is metronidazole given orally, 750 mg three times daily for 10 days (Pediatric dose: 35-50 mg/kg/day in three doses for 5 days) or iodoquinol given at 650 mg three times daily for 20 days. However, there have been reported cases of resistance. Trimethoprim-sulfamethoxazole (TMP-SMX) has also been found to be highly effective against *Blastocystis*. Nitazoxanide has been clinically tested on patients with blastocystosis, and was found to

resolve symptoms in 86% of patients after 3 days of administration.

Epidemiology

Blastocystis hominis has been reported virtually worldwide, with infections occurring most commonly in tropical, subtropical, and developing countries. Studies from developed countries have reported approximately 1.5 to 17.9% overall prevalence of *B. hominis*. All ages are affected, but symptomatic cases are more often found in children and in those with weakened immune systems. A prevalence of up to 11.6% was reported from Stanford University Hospital. Prevalence rates of 32.6 % and as high as 52.3% had been reported from China and Malaysia, respectively.

Occurrence of the parasite in temperate countries is generally associated with recent travel to the tropics and consumption of untreated drinking water. This indicates that infection is possibly through the oral route, and it is more likely to occur in crowded and unsanitary conditions. Outbreaks of *B. hominis* in day-care centers have been reported in Spain (5.3-10.3%), Brazil (34.7%), and Canada (13.4%).

In the Philippines, examination of 772 stools from consecutive patients at the Department of Parasitology, College of Public Health, University of the Philippines Manila, showed a prevalence of 20.7%, sometimes with concomitant infection with other intestinal parasites. Studies have also shown prevalence rates of 40.6% among food service workers in a tertiary hospital, and 23.6% among food handlers in selected school canteens in Manila. Stool surveys conducted by the Field Epidemiology Training Program of the Department of Health in Tapel, Gonzaga, Cagayan Valley, and Talavera, Nueva Ecija showed prevalence rates of 20% and 44%, respectively.

Some animals, like pig-tailed macaques, chickens, dogs, and ostriches may harbor

Blastocystis similar to those found in humans. Evidence has also shown that *Blastocystis* is present in house lizards and cockroaches, raising the possibility that food and water contaminated by fecal droppings of these “home visitors” may transmit *Blastocystis*.

In the Philippines, studies of 32 morphologically similar isolates from different hosts: 12 from humans, 12 from pigs, and 8 from chickens, using the restriction fragment length polymorphism (RFLP) analysis of small subunit rDNA (SSUrDNA), have shown extensive genomic polymorphism.

Prevention and Control

Available data on *B. hominis* indicate that the disease can be prevented by consuming safe drinking water. While food has not been fully implicated, provisions for sanitary preparation may be of value in efforts to prevent and control this infection. The cysts of *B. hominis* can survive up to 19 days in water at normal temperature, and have shown resistance to chlorine at the standard concentrations.

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Dientamoeba fragilis

Vicente Y. Belizario, Jr., Timothy M. Ting

Dientamoeba fragilis was first discovered by Wenyon in 1909 but was first described in the scientific literature by Jepps and Dobell in 1918. It remains neglected despite evidence supporting its pathogenicity. It has been identified in practically all regions of the world in which satisfactory iron-hematoxylin stained films have been carefully examined.

Parasite Biology

On the basis of electron microscopic, immunologic, and molecular phylogenetic findings, this protozoan, which was originally described as an ameba, is actually a flagellate with only the trophozoite stage known (Plate 2.15). The organism measures about 7 to 12 μm with one or two (rarely three or four) rosette-shaped nuclei. The nuclear membrane does not have peripheral chromatin, and the karyosome consists of four to six discrete granules. The cytoplasm may contain vacuoles with ingested

debris. No cyst stage has been identified. Except for the absence of a flagellum, this protozoan is closely related to and resembles *Trichomonas*.

D. fragilis lives in the mucosal crypts of the appendix, cecum and the upper colon. The exact life cycle is unknown, although several assumptions have been made from clinical data (Figure 2.13). Direct human to human transmission is probably via the fecal-oral route or via transmission of helminth eggs particularly that of *Enterobius vermicularis*. *Dientamoeba*-like mononucleated and binucleated forms have been observed in the lumen of *Enterobius* adults and eggs present in the intestines. More recently, stools from macaques, gorillas, and swine were found to carry *D. fragilis*, thus animal reservoirs may also be potential sources of human infections.

Pathogenesis and Clinical Manifestations

Dientamoeba fragilis does not invade tissues, but its presence in the intestines produces irritation of the mucosa with secretion of excess mucus and hypermotility of the bowel. Infections are usually asymptomatic. In symptomatic individuals, the onset of infection is usually accompanied by loss of appetite, colicky abdominal pain, and intermittent diarrhea with excess mucus, abdominal tenderness, a bloating sensation, and flatulence. Another common symptom, reported in 11% of the patients, was anal pruritus. This may partially be due to the co-infection with *Enterobius*. Peripheral eosinophilia can be observed in 50% of the cases. Chronic infection of this organism can mimic the symptoms of diarrhea-predominant irritable bowel syndrome (IBS), and some experts have suggested ruling out infection with this organism first before diagnosing a patient as having IBS.

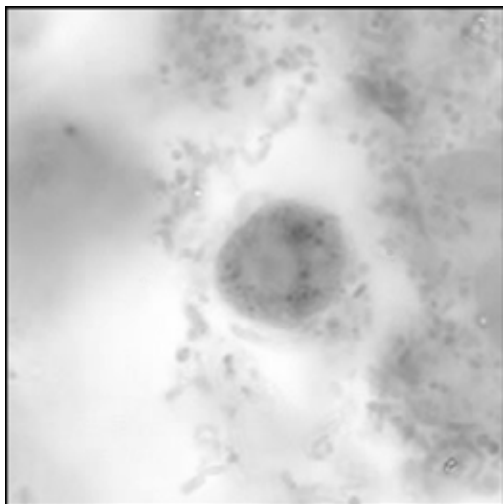


Plate 2.15. Binucleate forms of trophozoites of *Dientamoeba fragilis*, stained with trichrome (Accessed from www.dpd.cdc.gov/dpdx)

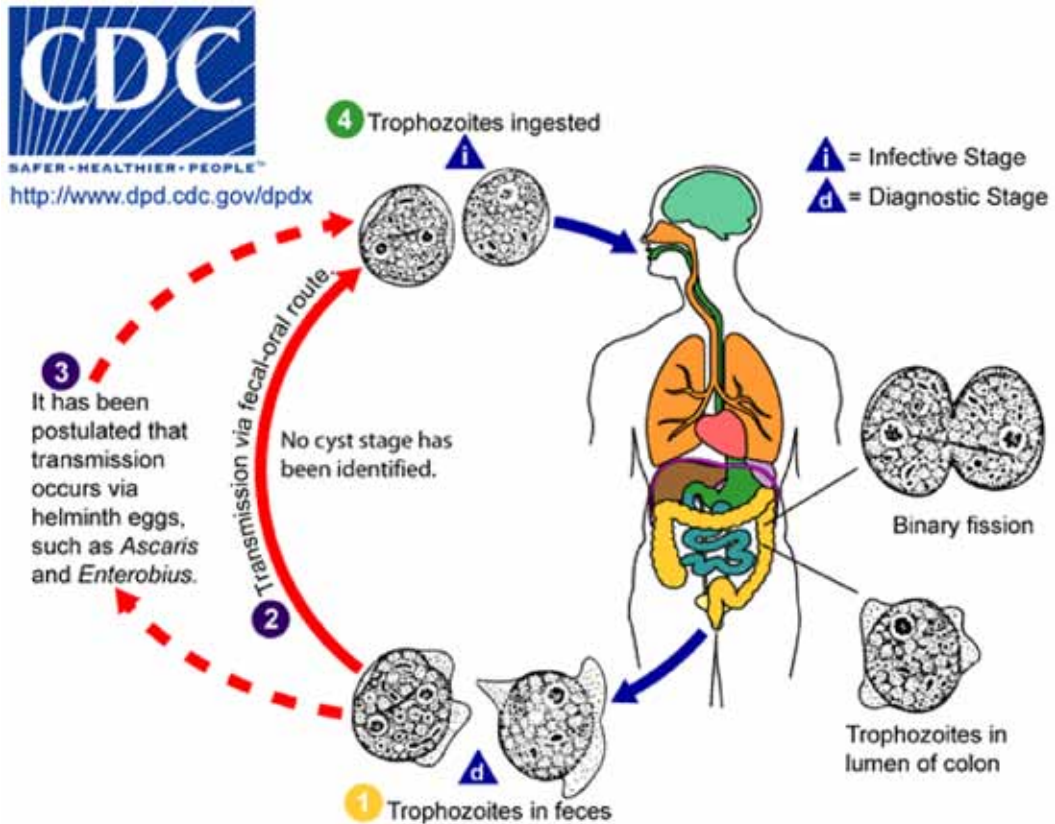


Figure 2.13. Life cycle of *Dientamoeba fragilis*
(Accessed from www.dpd.cdc.gov/dpdx)

Diagnosis

Diagnosis of this organism is by observation of binucleate trophozoites in multiple fixed and stained fresh stool samples. Fresh stool samples are necessary since the trophozoites degenerate after a few hours of stool passage. Multiple samples increase the sensitivity of detecting the organism. Unless the laboratory examiner is aware of the possibility that *D. fragilis* may be present in the fresh fecal films, the protozoan is easily overlooked. Purged stool specimens provide more suitable material for examination than the average formed stool. Even when formed, *D. fragilis* may be misdiagnosed as other amebae. This organism is not detected by stool concentration methods. Prompt fixation

of the fresh specimen with polyvinyl alcohol fixative or Schaudinn's fixative has been found to be helpful.

Treatment

Antimicrobial therapy is followed by resolution of symptoms and eradication of *D. fragilis*. Treatment is done with iodoquinol at 650 mg three times daily for 20 days. The pediatric dose is 40 mg/kg/day in three doses, also for 20 days. Tetracycline and metronidazole have also been found to be effective.

Epidemiology

The organism has a world-wide distribution with varying infection rates ranging from 0.4 to

as high as 42%. In contrast to many pathogenic protozoa, which have a high prevalence in developing countries, high prevalence rates of *D. fragilis* have been reported from developed countries with high sanitation standards. Using adequate culture techniques, the rates were as high as 18% in Israel, 36% in Holland, and 41.5% in Germany.

Prevention and Control

Specific recommendations for prevention and control cannot be made until there is more specific information concerning the method of transmission. Proper sanitation and disposal of human waste are essential.

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Plasmodium spp.

Vicente Y. Belizario, Jr., Carlos Miguel P. Perez

Malaria remains the leading parasitic disease that causes mortality worldwide. With 655,000 malaria-related deaths reported in 2010 and an estimated 3.3 billion people at risk for infection, the disease has been identified by the World Health Organization (WHO) as one of the three major infectious disease threats, along with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) and tuberculosis, which together cause more than 5 million deaths each year. Malaria leads to decreased social and economic productivity and contributes to a vicious cycle of disease and poverty. Young children and pregnant women are the population groups mostly affected by malaria. Chronic malaria leads to anemia, which is associated with impaired physical and mental growth and development in children. In pregnancy, anemia is a leading contributor to maternal morbidity and mortality, and is associated with risk of cardiac failure and adverse perinatal outcomes. Anemia from malaria is also exacerbated by anemia from concomitant helminth infections in both children and pregnant women.

In 2000, the United Nations (UN) adopted the Millennium Declaration, serving as a blueprint for the eradication of extreme poverty through eight quantifiable time-bound targets known as the Millennium Development Goals (MDGs) (Table 2.4). MDG 6 aims to reduce the burden of HIV/AIDS, malaria, and other diseases. The malaria component of MDG 6 includes reducing incidence and mortality rates of the disease, increasing insecticide-treated bed net coverage among children below 5 years of age and increasing anti-malarial coverage among children below 5 years of age.

Despite the high figures in mortality, the disease is curable if promptly and adequately

Table 2.4. Millennium development goals: eight goals for 2015

Millennium Development Goals	
1	Eradicate extreme poverty and hunger
2	Achieve universal primary education
3	Promote gender equality and women empowerment
4	Reduce child mortality
5	Improve maternal health
6	Combat HIV/AIDS, malaria, and other diseases
7	Ensure environmental sustainability
8	Develop a global partnership for development
Source: United Nations. General assembly, 56th session. Road map towards implementation of the united nations millennium declaration: report of the secretary-general (UN Document no. A/56/326). New York: United Nations, 2001.	

treated. The group of parasites causing malaria belongs to the genus *Plasmodium* that is transmitted by the bite of an infected female mosquito belonging to the genus *Anopheles*. The four species that are medically important to humans are *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. The first two are responsible for over 90% of all human malaria cases. More recently, *P. knowlesi* has been described in humans in the Philippines and most of Southeast Asia. *P. knowlesi*, considered the fifth human malaria parasite, is normally a parasite of long-tailed macaques (*Macaca fascicularis*), but humans working in nearby forest fringe pose great risk for infection. The first naturally acquired human infection was reported in 1965 in Sarawak, Malaysia; other foci of infection have been reported in Thailand and China as late as 2008. In the Philippines, the first reported case of *P. knowlesi* was described in 2006. Since then, the Research Institute for Tropical Medicine (RITM) has reported nine cases of mixed malaria infection,

positive for *P. knowlesi*. The life cycle of *P. knowlesi* is microscopically indistinguishable from *P. malariae*, and differentiation is only achieved through polymerase chain reaction (PCR) assay and molecular characterization.

These protozoans are pigment producers and are ameboid in shape, with some being more ameboid than the others. Their asexual cycle occurs in humans, the vertebrate and intermediate host, while the sexual cycle occurs in the *Anopheles* mosquito, the invertebrate and definitive host.

Parasite Biology

Various processes comprise the life cycle (Figure 2.14) of the parasite. The asexual cycle

in humans consists of schizogony, which leads to the formation of merozoites, and gametogony, which leads to the formation of gametocytes.

The sexual cycle in the mosquito involves sporogony, which leads to the formation of sporozoites. The life cycles of all human species of malaria are similar. The infected female *Anopheles* mosquito bites and sucks blood from the human host. In the process, salivary fluids containing sporozoites are also injected. These sporozoites, the infective stage of the parasite, are immediately carried to the liver and enter the parenchymal cells. The parasites then commence exo-erythrocytic schizogony, which produces the merozoites in varying duration and amounts, depending

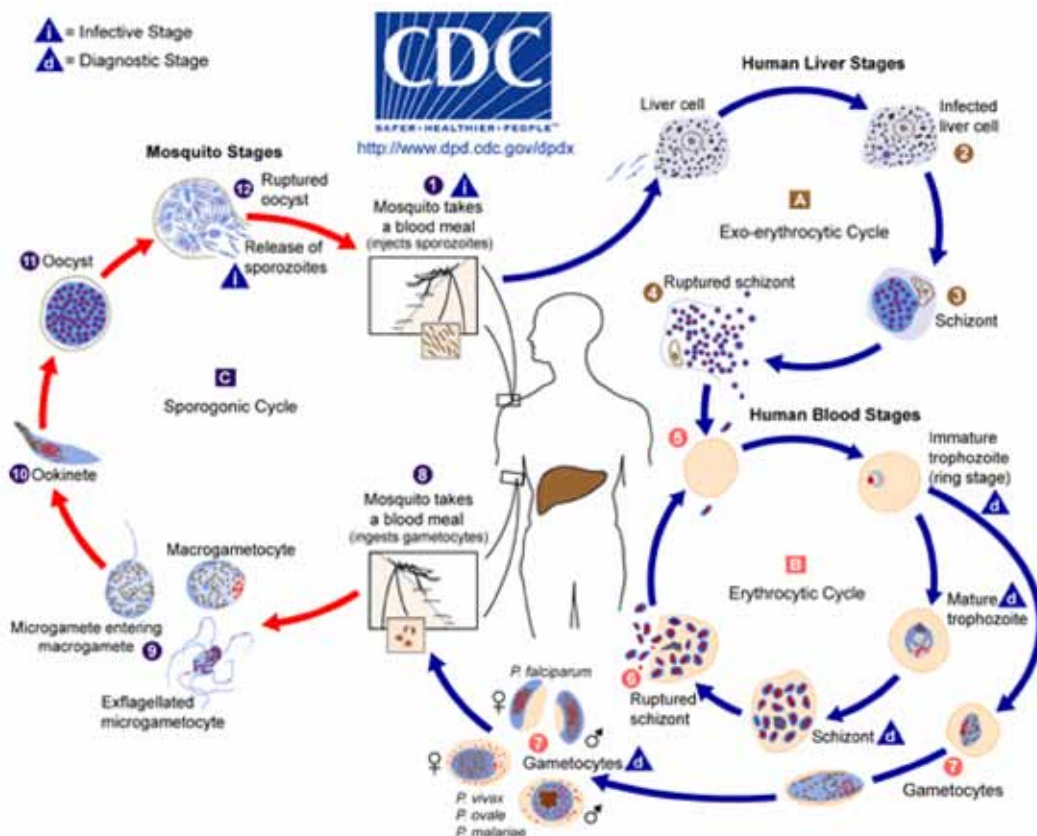


Figure 2.14. Life cycle of *Plasmodium* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

on the species. Merozoites proceed to the peripheral blood to enter the erythrocytes. Some merozoites of *P. vivax* and *P. ovale* re-invade the liver cells forming hypnozoites, while the other species do not. These dormant exo-erythrocytic forms may remain quiet for years. Within the red blood cell, the merozoite grows as a ring form developing into a trophozoite. The trophozoite has an extended cytoplasm and a large chromatin mass which further divides to form more merozoites within schizonts. The merozoites of *P. falciparum* develop in the parasitophorous vacuolar membrane (PVM) within the mature red cells and modify the structural and antigenic properties of these cells. The parasites feed on the hemoglobin resulting in the production of pigment known as hemozoin. Soon after, the erythrocytes rupture and the merozoites are released into the blood, ready to enter new erythrocytes. This asexual cycle is synchronous, periodic, and species-determined.

Some merozoites develop into microgametocytes (male) or macrogametocytes (female) which are picked up by feeding female mosquitoes for completion of the life cycle. In the gut of the mosquito, the male gametocytes exflagellate and produce eight sperm-like microgametes which may fertilize the female macrogamete to form a zygote. The zygote becomes motile and penetrates the mosquito's

gut as an ookinete, which then develops into an oocyst. The oocyst grows and produces sporozoites, which escape from the oocyst and enter the salivary glands of the mosquito. These sporozoites may be injected into another human host when the mosquito takes a blood meal. The entire developmental cycle in the mosquito takes 8 to 35 days, depending to some extent on ambient temperature.

Morphologically, the early trophozoite form is ring-shaped with a red chromatin dot and a scant amount of blue cytoplasm when stained with Giemsa or Wright's stain. The trophozoite form has a large chromatin mass and a prominent ameboid cytoplasm, which is spread through the erythrocyte. The parasite develops into a schizont when the chromatin has divided into two or more masses of chromatin with small amounts of cytoplasm, the so-called merozoites. The number of merozoites is species dependent. Clumps of pigment accumulate in the middle of a mature schizont.

The gametocyte stage fills the entire red blood cell and is characterized by a large chromatin mass and a blue cytoplasm with pigment. It is round to banana-shaped. The microgametocyte has a lighter blue cytoplasm, while the cytoplasm of the macrogametocyte is a darker blue. Species identification depends on various characteristics of these stages of development as described in Table 2.5.

Table 2.5. Comparison of morphological features of malaria parasites

Parameter	<i>Plasmodium</i> species			
	<i>P. falciparum</i> (malignant tertian)	<i>P. vivax</i> (benign tertian)	<i>P. ovale</i> (benign tertian)	<i>P. malariae</i> (quartan)
Infected red blood cells (RBC)	Normal: multiple infection of RBC very common	Larger than normal, pale, often bizarre; Schüffner's dots are often present; multiple infection of RBC not uncommon	Somewhat larger than normal, often with fringed or irregular edge, and oval in shape; Schüffner's dots appear even with younger stages; stains more readily and deeply than in <i>P. vivax</i>	Larger than normal, pale, often bizarre; Schüffner's dots are often present; multiple infection of RBC not uncommon

Parameter	<i>Plasmodium species</i>			
	<i>P. falciparum</i> (malignant tertian)	<i>P. vivax</i> (benign tertian)	<i>P. ovale</i> (benign tertian)	<i>P. malariae</i> (quartan)
Small trophozoite	Same as <i>P. vivax</i> but with small threadlike blue cytoplasmic circle with one or two small red chromatin dots; double chromatin common; marginal forms common	Signet-ring form with heavy red dot and blue cytoplasmic ring	Small, darker in color, and generally more solid than those of <i>P. falciparum</i> ; Schüffner's dots regularly present in almost 100% of infected cells	Same as <i>P. vivax</i> but with blue cytoplasmic circle, smaller, thicker and heavier
Growing trophozoite	Remains in ring form but grows resembling small trophozoite of <i>P. vivax</i> in size; usually the oldest asexual stage seen in peripheral blood	Like small trophozoite, as above, with increased cytoplasm and ameboid activity; small-yellowish brown pigment granules in cytoplasm, increasing with age of parasite	Resembles closely same stage of <i>P. malariae</i> but is considerably larger; pigment is lighter and less conspicuous	Chromatin rounded or elongated; cytoplasm compact or in narrow band across cell; dark brown granules may have peripheral arrangement
Large trophozoite	Seldom present	Large mass of chromatin; loose, irregular, or close compact cytoplasm with increasing amount of fine brown pigment; parasite fills cell in 30 to 40 hours	Seldom present	Chromatin often elongate, indefinite in outline; cytoplasm dense, compact, in rounded oblong or band forms; pigment granules larger, darker than <i>P. vivax</i> parasite fills cells frequently
Schizont (presegmenting)	Not present	Chromatin divided; cytoplasm shows varying degrees of separation into strands and particles; pigment collects in parts of the parasite	About 25% of infected cells are definitely oval shaped: usual picture is that of a round parasite in the center of an oval cell; many cells with indefinite fringed outline; pigment lighter and less coarse than in <i>P. malariae</i>	Same as <i>P. vivax</i> except parasite is smaller, shows less chromatin division, more delayed clumping of pigment
Schizont (mature)	Rarely present; 8-24 merozoites; smaller than other species	12-24 merozoites; pigment in one to two clumps; parasite almost fills enlarged cells	Usually eight merozoites arranged around a central block of pigment	6-12 (average of 8-10) merozoites in rosette form; parasite almost fills cell

Pathogenesis and Clinical Manifestations

The interval from sporozoite injection to detection of parasites in the blood is referred to as the pre-patent period. Depending on the

species involved, this may range from 11 days to 4 weeks. The average pre-patent period for *P. falciparum* is 11 to 14 days, for *P. vivax*, 11 to 15 days, for *P. ovale*, 14 to 26 days, and 3 to 4 weeks for *P. malariae*. The incubation

Parameter	<i>Plasmodium</i> species			
	<i>P. falciparum</i> (malignant tertian)	<i>P. vivax</i> (benign tertian)	<i>P. ovale</i> (benign tertian)	<i>P. malariae</i> (quartan)
Gametocyte	Present in peripheral blood stream, similar to <i>P. vivax</i> ; crescent or sausage shape	Microgametocyte: light red to pink chromatin, diffuse, central; gives tint to light blue cytoplasm; yellowish brown pigment throughout cytoplasm; usually round and about the size of normal RBC Macrogametocyte: small, compact, dark red eccentric chromatin; cytoplasm dark blue, no vacuoles; abundant dark brown pigment scattered throughout the cytoplasm	Distinguished from <i>P. malariae</i> by size of infected cells and by Schüffner's dots; less easy to differentiate from <i>P. vivax</i>	Same as <i>P. vivax</i> except smaller; fills or almost fills cells
Stages in peripheral blood	Ring forms and gametocytes; other stages rare	All stages present	All stages present	All stages present
Length of asexual cycle	48 hours or less	48 hours	48 hours	72 hours
Note: <i>P. knowlesi</i> is microscopically indistinguishable from <i>P. malariae</i> .				

period, the time between sporozoite injection and the appearance of clinical symptoms, is typically 8 to 40 days, depending again on the involved species. For *P. falciparum*, it lasts an average of 8 to 15 days, for *P. vivax*, 12 to 20 days, for *P. ovale*, 11 to 16 days, and for *P. malariae*, 18 to 40 days. The incubation period may range from 9 days to 3 years, depending on the parasite strain, the dose of sporozoites inoculated, the immune status of the host, and the host's malaria chemoprophylaxis history. Partial or incomplete prophylaxis may prolong the incubation period several weeks after termination of medication. Any person who has traveled to a malaria-endemic area must be considered at risk of developing malaria up to 2 years and even longer upon leaving the area.

There are no absolute clinical features of malaria except for the regular paroxysms of fever

with the associated asymptomatic intervals. Prodromal symptoms may include: a feeling of weakness and exhaustion, a desire to stretch and yawn, aching bones, limbs, and back, loss of appetite, nausea and vomiting, and a sense of chilling. At the onset, symptoms may include malaise, backache, diarrhea, and epigastric discomfort. The classical malaria paroxysms have three stages: the cold stage, the hot stage, and the sweating stage. The cold stage starts with a sudden inappropriate feeling of coldness and apprehension. Mild shivering quickly turns to violent teeth chattering and shaking of the entire body. Although the core temperature is high or may be rising quickly, there is intense peripheral vasoconstriction. The patient may vomit and febrile convulsions may ensue at this stage in young children. These rigors last for 15 to 60 minutes after which the shivering

ceases, and the hot stage or flush phase begins. The patient becomes hot and manifests with headache, palpitations, tachypnea, epigastric discomfort, thirst, nausea, and vomiting. The temperature may reach a peak of 41°C or even more. The patient may become confused or delirious, and the skin may be notably flushed and hot. This phase lasts from 2 to 6 hours. In the sweating stage, defervescence or diaphoresis ensues with the patient manifesting with profuse sweating. The temperature lowers over the next 2 to 4 hours, and symptoms diminish accordingly. The total duration of a typical attack is 8 to 12 hours. The classic periodicity of attacks develops only if the patient is left untreated until the time when the life cycle phases become synchronized and sufficient numbers of red blood cells containing schizonts rupture at about the same time. The interval between attacks is determined by the length of the erythrocytic cycle. For *P. falciparum*, it is 48 hours. For *P. vivax* and *P. ovale*, paroxysms occur on alternate days. For *P. malariae*, they occur every 72 hours, causing paroxysms on days 1 and 4, hence the term, quartan malaria. Due to the lack of an exoerythrocytic stage in *P. knowlesi*, fever follows a quotidian pattern, or is noted to be non-relapsing.

The five species also differ in the age of infected erythrocytes. The non-falciparum species infect erythrocytes only of a certain age: *P. vivax* and *P. ovale* infect only young red blood cells, while *P. malariae* infects only aging cells. This limits the number of red blood cells that can be parasitized to less than 3% of all erythrocytes. *P. falciparum*, as well as *P. knowlesi*, may infect erythrocytes of all ages. As the infected erythrocytes rupture, more falciparum malaria parasites are released to infect more red blood cells. The severity of complications and mortality increase as the level of parasitemia increases. The course and severity of the attack of malaria depend on the species and the strain of the infecting parasite; therefore, geographical origin of infection plays a major role in disease

distribution. They also depend on the age, genetic constitution, state of immunity, general health and nutritional status of the host, and on any chemoprophylaxis or chemotherapy previously used.

There may be a tendency to recrudescence or relapse over a period of months to several years. Recrudescence is the renewal of parasitemia or clinical features arising from persistent undetectable asexual parasitemia in the absence of an exo-erythrocytic cycle. Relapse is renewed asexual parasitemia following a period in which the blood contains no detectable parasites (Figure 2.15). Relapses, which occur with vivax and ovale malaria, result from the reactivation of hypnozoite forms of the parasite in the liver. Cold, fatigue, trauma, pregnancy, and infections including intercurrent falciparum malaria may precipitate reactivation.

The pathological processes in malaria are the result of the erythrocytic cycle. Once the merozoites of *P. falciparum* invade the erythrocytes, the cells reduce their deformability, the degree of which is directly proportional to parasite maturity. This reduction in deformability is due to changes in the red blood cell cytoskeleton and the increase in membrane stiffness and cytoplasmic viscosity. In the course of invasion, electron-dense sub-membranous structures appear and enlarge. These become the so-called “knobs” which are important in cytoadhesion. They contain several proteins such as rosetins, riffsins, histidine-rich proteins (HRP), and the *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP-1), which is the most adhesive protein among the knobs. PfEMP-1 is encoded by a multi-gene family termed *var* and is clonally variant enabling it to evade specific immune responses. Rosettins and PfEMP-1 are the ligands for rosette formation. They adhere to parasitized and non-parasitized cells as well as blood platelets. In more recent studies, it has been suggested that febrile temperatures induce the cytoadherence of the ring-staged

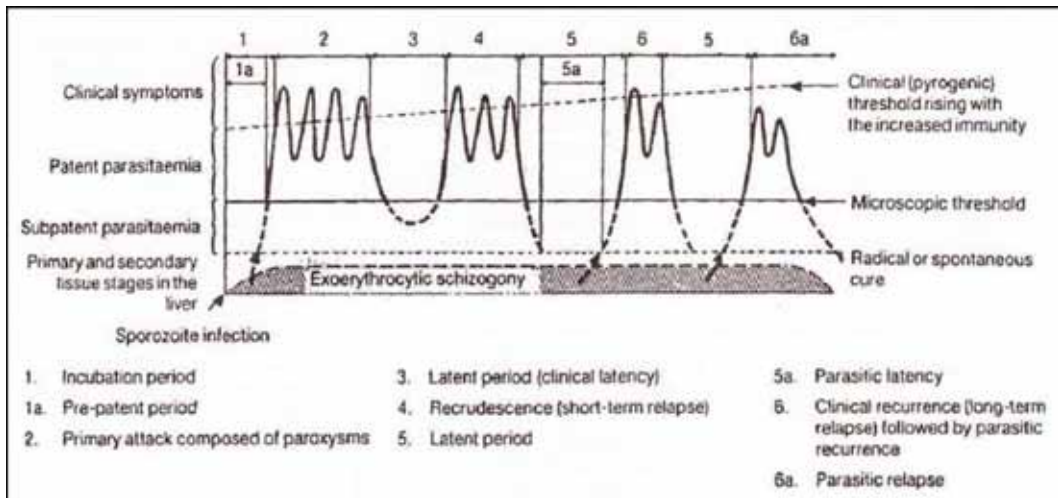


Figure 2.15. Diagram of the course of malaria infections showing the primary attack, relapses, and recrudescence (From World Health Organization. Chemotherapy of malaria and resistance to antimalarials: report of a WHO scientific group. Technical report series no. 529. Geneva: World Health Organization; 1973.)

P. falciparum erythrocytes, and that the factor responsible for this heat-induced cytoadherence is PfEMP-1. HRP, on the other hand, localizes to the cytoadherence ligands making the adhesion more effective.

Infected erythrocytes also undergo altered membrane transport mechanisms. The hemoglobin is digested forming the pigment hemozoin, and variant strain-specific neoantigens are expressed. The soluble antigens of *P. falciparum* are potent inducers of pro-inflammatory as well as anti-inflammatory cytokines from monocytes and macrophages. Glycosylphosphatidyl inositol (GPI) moieties that are seen covalently linked to the surface antigens of these protozoans act like the endotoxin of gram-negative bacteria, lipopolysaccharide (LPS). They stimulate the monocytes to release tumor necrosis factor (TNF) or cachexin, which is implicated as the cause of malarial fever. The fever, febrile paroxysms, headache, various aches and pains, and prostration, which are the more familiar symptoms of an acute malarial attack, are probably the result of the release of these

cytokines at the time of schizont rupture. The combination of altered red cell surface membranes and the host's immunological response to the parasite antigens bring about the pathologic changes such as alteration in regional blood flow in the vascular endothelium, altered biochemistry, anemia, and tissue and organ hypoxia. Other destructive tissue processes include increased capillary permeability which allows fluid to leak into surrounding tissues, and congestion in blood vessels resulting in tissue infarction and necrosis.

In severe forms of malaria, impairment of consciousness and other signs of cerebral dysfunction, such as delirium and generalized convulsions, are commonly observed. Other manifestations are severe hemolytic anemia with a hematocrit less than 20%, hemoglobin levels less than 7 g/dL and hyperbilirubinemia with levels more than 50 mmol/L (Table 2.6).

Cerebral malaria generally manifests with diffuse symmetric encephalopathy. Other signs and symptoms include retinal hemorrhage, bruxism (fixed jaw closure and teeth grinding), and mild neck stiffness. Pouting may occur or

Table 2.6. Clinical features and laboratory findings in severe malaria infection

Clinical features	Laboratory results
<ul style="list-style-type: none"> • Impaired consciousness or coma • Prostration • Failure to feed • Multiple convulsions* • Deep breathing • Respiratory distress • Circulatory collapse or shock (systolic blood pressure below 50 mmHg in children) • Clinical jaundice • Other evidence of vital organ dysfunction • Abnormal spontaneous bleeding • Pulmonary edema (radiological) 	<ul style="list-style-type: none"> • Hypoglycemia (blood glucose <2.2 mmol/L or <40 mg/dL) • Metabolic acidosis (plasma bicarbonate <15 mmol/L) • Severe normocytic anemia (Hb <5 g/dL, packed cell volume <15%) • Hemoglobinuria • Hyperparasitemia (>2% or 100 000/μL in low intensity transmission areas or >5% or 250,000/μL in areas of high stable malaria transmission intensity) • Hyperlactatemia (lactate >5 mmol/L) • Renal impairment (serum creatinine >265 μmol/L)
*more than two episodes in 24 hours	Note: Severe <i>P. falciparum</i> infection, one or more clinical feature and laboratory finding
Source: World Health Organization. Management of severe malaria: a practical handbook. Geneva: World Health Organization; 2000.	

a pout reflex may be elicited by stroking the sides of the mouth. Lumbar tap usually reveals a normal to elevated opening pressure, clear cerebrospinal fluid (CSF) with fewer than 10 leukocytes/mL, and slightly elevated protein and CSF lactic acid concentration. If left untreated, symptoms progress to persistent coma and death. The neurological complications, once promptly and adequately treated, are reversible and a majority of the patients make a complete recovery.

Respiratory findings are also a major feature of severe malaria. Altered pulmonary function is common, and it includes air flow obstruction, impaired ventilation and gas transfer, and increased pulmonary phagocytic activity. In African children, pneumonitis from sequestered, parasitized RBC and inflammatory cells are seen in postmortem pulmonary vasculature, while in adults, non-cardiogenic pulmonary edema and acute pulmonary distress syndrome (ARDS) may be observed. There is a high mortality rate (over 80%) when pulmonary edema develops in a patient with severe malaria. Factors which predispose to pulmonary edema include hyperparasitemia, renal failure, and pregnancy.

The incidence of acute renal failure (ARF) reaches up to 60% of patients with severe falciparum malaria, with more males being

affected. Malaria ARF is defined as having a serum creatinine of more than 265 mmol/L (3 mg/dL) and a 24-hour urine output of less than 1 ml/kg/hr, despite rehydration, in patients with asexual forms of the parasite present in their peripheral blood smear. The patient may also present with hyperkalemia and hyperuricemia earlier in the course. The cytoadherence, rosette formation, and sequestration of parasitized erythrocytes lead to a decrease in tissue perfusion resulting in decreased renal blood flow. The increase of TNF in tubular epithelial cells leads to inflammatory cell infiltration in the interstitium and altered tubular transport, which result in tubular damage and dysfunction. The presence of GPI and other falciparum malaria antigens lead to release of cytokines and mediators that decrease the systemic vascular resistance and increase renal vascular resistance. All these changes eventually lead to acute tubular necrosis causing acute renal failure.

Malaria in pregnancy increases the risk of maternal death, maternal anemia, intrauterine growth retardation, spontaneous abortion, stillbirth, and low birth weight associated with risk for neonatal death. Non-immune pregnant women are susceptible to all complications associated with severe malaria such as cerebral malaria, hypoglycemia, and pulmonary edema.

For partially immune pregnant women, especially primigravid, severe anemia may develop but the other complications of severe malaria are unlikely to occur. *Falciparum* malaria may induce uterine contractions, thus may push the patient to premature labor. In severe malaria, the prognosis of the fetus is poor.

Falciparum malaria in a young child is considered a medical emergency for it can be rapidly fatal. The initial symptoms may be atypical and difficult to recognize, but within hours, life-threatening complications may start to occur. The most common complications of severe malaria in children are cerebral malaria, severe anemia, respiratory distress, and hypoglycemia. Children with severe malaria most commonly present with seizures. These convulsions are common before or after the onset of coma and are significantly associated with neurologic sequelae. Opisthotonos may also be observed in some children. As much as 10% of children who survive cerebral malaria will develop sequelae such as hemiparesis, cerebellar ataxia, speech disorders, generalized spasticity, or some behavioral disturbances (Table 2.7).

Not everyone infected with the malaria parasite becomes seriously ill or dies. In areas where endemicity is stable, repeated exposures to the parasite lead to specific immunity. This restricts occurrence of serious problems in young children, while older patients have relatively mild febrile illness. In people who are exposed to malaria for the first time, possible outcomes may range from apparent resistance to death. Any resistance, therefore, is nonspecific. It also does not necessarily depend on prior exposure to malaria and may be either acquired or innate. Poor prognostic factors in *falciparum* malaria include hyperparasitemia defined as a peripheral count more than 250,000/ μ L or more than 5% of the RBCs infected, and the presence of mature or immature schizonts in a peripheral blood smear. It has been shown that a peripheral count of 10% or more of red blood cells infected has a mortality rate of 50%, particularly in non-immune cases, despite treatment. The clinical indicators of poor prognosis include deep coma, absence of corneal light reflex, respiratory distress (acidosis), circulatory collapse, decerebrate or decorticate rigidity, opisthotonos, and age

Table 2.7. Comparison of sign and symptoms of severe malaria in adults and children

Sign or symptom	Adults	Children
History of cough	Uncommon	Common
Convulsions	Common	Uncommon
Duration of illness	5-7 days	1-2 days
Resolution of coma	2-4 days	1-2 days
Neurological sequelae	<5%	>10%
Jaundice	Common	Uncommon
Pretreatment hypoglycemia	Uncommon	Common
Pulmonary edema	Uncommon	Common
Renal failure	Common	Uncommon
CSF opening pressure	Usually normal	Usually raised
Respiratory distress (acidosis)	Sometime	Common
Bleeding/clotting disturbances	Up to 10%	Rare
Abnormal brainstem reflex (e.g., oculovestibular, oculocervical)	Rare	More common

Source: World Health Organization. Management of severe malaria: a practical handbook. Geneva: World Health Organization; 2000.

below 3 years. Other laboratory indicators of poor prognosis include blood glucose <2.2 mmol/L, raised venous lactic acid (>5 mmol/L), more than three-fold increase in serum enzymes (aminotransferases), hemoglobin concentration less than 5g/dL, blood urea more than 60 mg/dl, serum creatinine more than 265 mmol/L, peripheral polymorphonuclear leukocytes with visible malaria pigment (>5%), low antithrombin III levels, and very high plasma concentrations of TNF.

Diagnosis

Prompt and adequate diagnosis of malaria is necessary for the disease to be managed effectively, thus preventing the life threatening complications. Though malaria may present with the classic paroxysms of fever with asymptomatic intervals, initial symptoms are non-specific and are not reliable in clinching the diagnosis. In fact, treatment based on clinical findings alone usually results in unnecessary and irrational drug use.

Microscopic identification of the malarial parasites in thick and thin blood smears stained with Giemsa or Wright's stain is still important in making the definitive diagnosis and remains as the gold standard. Specimens may be taken any time and all blood stages of the parasite may be found. In falciparum malaria, only the ring forms (Plate 2.16) may be found, but 10 days after the symptoms begin, gametocytes may be found as well. Although there are no standard recommendations on how often the blood smears should be taken in order to diagnose malaria, obtaining smears every 6 to 8 hours is usually appropriate. This may have to be continued until a diagnosis of malaria is made or until malaria can be confidently ruled out. When malaria is a serious condition, this may require repeated testing for several days in order to demonstrate a positive result. Even after the diagnosis of malaria has been made, peripheral blood smears should still be obtained to monitor the response to treatment. In individuals who

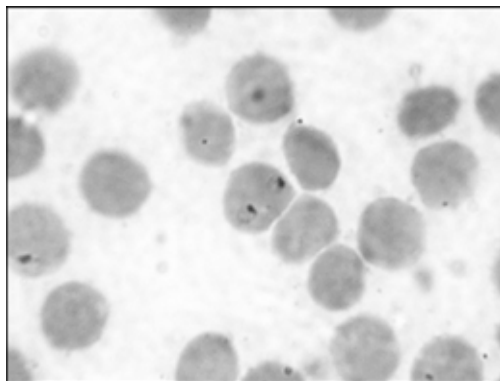


Plate 2.16. *Plasmodium falciparum* ring forms
(Courtesy of the Department of Parasitology,
UP-CPH)

are not seriously ill, monitoring once daily may be sufficient. Seriously ill patients should be monitored two to three times daily until significant improvement occurs. Monitoring should be continued until there is clearance of parasitemia.

Although microscopic diagnosis is the established diagnostic method, technical and personnel requirements often cannot be met, particularly facilities in the periphery of the health care system. This has led to the introduction of the malaria rapid diagnostic tests (RDTs). These tests make use of immunochromatographic methods in order to detect *Plasmodium*-specific antigens in a finger prick blood sample. Currently, the antigens being targeted by these RDTs include: histidine rich protein II (HRP II), which is a water soluble protein produced by trophozoites and young gametocytes of *P. falciparum*; *Plasmodium* lactate dehydrogenase (pLDH), which is produced by both sexual and asexual stages and can distinguish between *P. falciparum* and non-*P. falciparum*, but not among the non-*P. falciparum* species; and *Plasmodium* aldolase, an enzyme in the parasite glycolytic pathway expressed by the blood stages of all *Plasmodium* species. Together with HRP II, *Plasmodium* aldolase has been used in a combined immunochromatographic test targeting the panmalarial antigen (PMA).

These tests can be performed in 15 to 30 minutes without the use of electricity, special equipment, or any training in microscopy, and most kits have more than 90% specificity. More recent studies have shown that test kits based on HRP II have a sensitivity and specificity of 92.5% and 98.3% respectively, while kits based on the pLDH antigen have a lower sensitivity (88.5%) albeit a higher specificity at 99.4%. The use of RDTs can be easily taught to village health workers and the results can likewise be easily interpreted. The main disadvantages of RDTs compared to microscopy are: the lack of sensitivity at low levels of parasitemia; the inability to quantify parasite density; the inability to distinguish among *P. vivax*, *P. ovale*, and *P. malariae*, as well as sexual and asexual stages; the persistently positive tests (for some antigens) despite parasite clearance following chemotherapy; and the relatively higher cost per test.

In recent studies conducted in various areas of the Philippines to describe the validity of a few specific malaria RDT kits, results showed sensitivity and specificity levels below the WHO recommended ideal of 95% and 90%, respectively. Reasons for these findings could be manufacturer-related problems, the instability of the substances used in the diagnostic technique to varying environmental conditions such as extremes of temperature and humidity, and user-related problems. Quality assurance of these malaria RDT kits is, therefore, necessary before they are deployed on a larger scale in remote and rural areas. More recent studies are now concentrating on quality assurance of these tests and on identifying the factors which may affect RDT performance in the field.

Malaria can also be diagnosed serologically but presently available methods are not capable of making a definitive diagnosis of acute malaria. Available serologic tests like indirect hemagglutination (IHA), indirect fluorescent antibody test (IFAT), and enzyme-linked immunosorbent assay (ELISA) cannot

differentiate between current and past infections and are therefore most helpful in epidemiologic studies. Current studies are using PCR to significantly enhance microscopic diagnosis of malaria especially in cases of low parasitemia and in cases of mixed infection.

Treatment

Antimalarial drugs have selected actions on the different phases of the life cycle of the malaria parasite. These drugs may be classified into causal prophylactic drugs, which prevent the establishment of the parasite in the liver, and blood schizonticidal drugs, which attack the parasite in the red blood cell, preventing or terminating the clinical attack. Tissue schizonticides act on pre-erythrocytic forms in the liver. Gametocytocidal drugs destroy the sexual forms of the parasite in the blood. Some drugs are hypnozoitocidal or antirelapse drugs, which kill the dormant forms in the liver. Sporonticidal drugs inhibit the development of the oocysts on the gut wall of the mosquito, which has fed on a gametocyte carrier so that the mosquito cannot transmit the infection.

The main uses of antimalarial drugs are: (a) protective (prophylactic), (b) curative (therapeutic), and (c) preventive. Drugs for prophylaxis are used before the infection occurs or before it becomes evident, with the aim of preventing either the occurrence of the infection or any of its symptoms. A blood schizonticidal drug may have minimal effects on parasites growing in the liver, but if it is still present in the blood when the merozoites leave the liver and invade the blood cells for the first time, it will effectively prevent symptomatic malaria. Curative or therapeutic use refers to action on the established infection, which involves the use of blood schizonticidal drugs for the treatment of the acute attack and in the case of relapsing malaria, radical treatment of the dormant liver forms. Prevention of transmission means the deterrence of infection of mosquitoes with the use of gametocytocidal drugs to attack the

gametocytes in the blood of the human host. It also means the interruption of the development of the sporogonic phase in the mosquito when it feeds on the blood of an infected person who has been given the appropriate sporonticidal compound.

Chloroquine was the mainstay of antimalarial treatment for the last 50 years. Because of emergence of multidrug-resistant (MDR) strains, subsequent chloroquine use has been rendered ineffective against falciparum malaria, and the current DOH Malaria Control Program (MCP) recommends the use of artemisinin-based combination therapies (ACTs) for severe and uncomplicated falciparum malaria, replacing the chloroquine plus sulfadoxine-pyrimethamine combination. The following drug combinations are recommended: artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, and artesunate together with sulfadoxine-pyrimethamine. For severe malaria, parenteral antimalarial treatment should be started without delay after rapid clinical assessment and confirmation of the diagnosis. The following antimalarial drugs are recommended: artesunate intravenous (IV) injection or intramuscular (IM) injection, quinine IV or IM, or artemether IM. In a placebo-controlled trial, patients with severe malaria who could not be treated orally and where access to IM and IV treatment was unavailable, a single artesunate suppository at the time of referral reduced the risk of death or permanent disability.

Current guidelines also recommend the use of gametocytocidal drugs to reduce transmission. Seen in the context of malaria elimination, the use of primaquine 0.75 mg base/ kg body weight single oral dose demonstrates an added benefit to artemisinins in eliminating gametocytes. The addition of a single dose of primaquine to current ACTs is therefore recommended provided that the risk for hemolysis in G6PD deficient patients is considered. Moreover, primaquine should not

be given in pregnancy and in children less than 4 years of age.

In contrast with falciparum malaria, vivax malaria remains sensitive to chloroquine. Clinical studies and extensive *in vitro* observations have shown that *P. vivax* is still generally sensitive to chloroquine, although resistance is prevalent and increasing in Indonesia, Peru, and Oceania. Moreover, vivax malaria is sensitive to all other antimalarial drugs albeit slightly less sensitive to artesunate plus sulfadoxine-pyrimethamine. The asexual stage of *P. vivax* remains susceptible to primaquine; therefore, combination treatment with chloroquine and primaquine affords blood stage and liver stage treatment, respectively. Often referred to as radical treatment, the use of primaquine, together with chloroquine, allows for the prevention of relapse in vivax malaria. In comparison with no primaquine treatment, the risk of relapse decreases for every additional mg/kg of primaquine given. Repeated vivax malaria relapses are debilitating at any age, hence they must be prevented. At least a 14-day course of primaquine is needed for the radical treatment of *P. vivax*.

Resistance of *P. malariae* and *P. ovale* to antimalarials is not well characterized, and infections with these species are still considered sensitive to chloroquine. The treatment for relapsing fever caused by *P. ovale* is similar to that of vivax malaria (i.e., chloroquine and primaquine). In the case of mixed malarial infections, ACTs remain the mainstay treatment. Moreover, the use of artemisinin-based compounds and a partner drug with a long half-life (i.e., artesunate plus amodiaquine and dihydroartemisinin plus piperaquine) has been effective against in chloroquine-resistant vivax malaria. Radical treatment with primaquine should also be considered in cases of confirmed *P. vivax* and *P. ovale* infections.

Artemisinin and its derivatives (*Qinghaosu* derivatives), artesunate, and artemether produce rapid clearance of parasitemia and rapid

resolution of symptoms. Because artemisinins are rapidly eliminated in the body, the duration of treatment is dependent on the partner drug being short acting or long acting. When partnered with rapidly eliminated drugs (tetracyclines and clindamycin), a 7-day course of treatment is usually required. Treatment duration can be reduced to 3 days when artemisinins are given in combination with slowly eliminated drugs such as mefloquine and amodiaquine. An additional advantage from a public health standpoint is the ability of artemisinins to reduce gametocyte carriage, thus reducing the transmission of malaria. This form of malaria control is particularly useful in areas of low to moderate endemicity.

Quinine sulfate plus doxycycline or clindamycin serves as the second line drug when artemisinins (e.g., IV artesunate) are unavailable or when there is failure to respond to artemisinin therapy. The tetracyclines and clindamycin are known to be effective antimalarials, although they kill the parasite rather slowly. Quinine has the disadvantage of producing toxic side effects such as cardiotoxicity and cinchonism, characterized by tinnitus, headache, and blurring of vision. Also, rapid administration of quinine is unsafe. Each dose of parenteral quinine must be administered as a slow rate-controlled infusion, and electrocardiographic (ECG) monitoring and frequent assessment of vital signs are required if quinines are used. Due to the risk of congenital defects, tetracycline is contraindicated in pregnant women and children below 8 years. Rather, quinine plus clindamycin taken for 7 days remains the antimalarial of choice in pregnancy.

The problem of drug resistance involves mainly chloroquine plus sulfadoxine-pyrimethamine and certain strains of *P. falciparum*. Such strains are often MDR. Asexual parasites are normally cleared from the blood three days after the start of treatment and are definitely cleared 6 or 7 days after start

of therapy. Resistance of a parasite to drugs is graded according to the patterns of asexual parasitemia after initiation of treatment (Figure 2.16). RI is the mildest form of resistance which is characterized by initial clearance of parasites but recrudescence occurs within a month after the start of treatment. It can be classified as either early, when clearance occurs for the first 48 hours and recrudescence takes place within the first 14 days after start of treatment, or late when there is also clearance within the first 48 hours and recrudescence occurs within the 14th to the 28th day from the start of treatment. RII

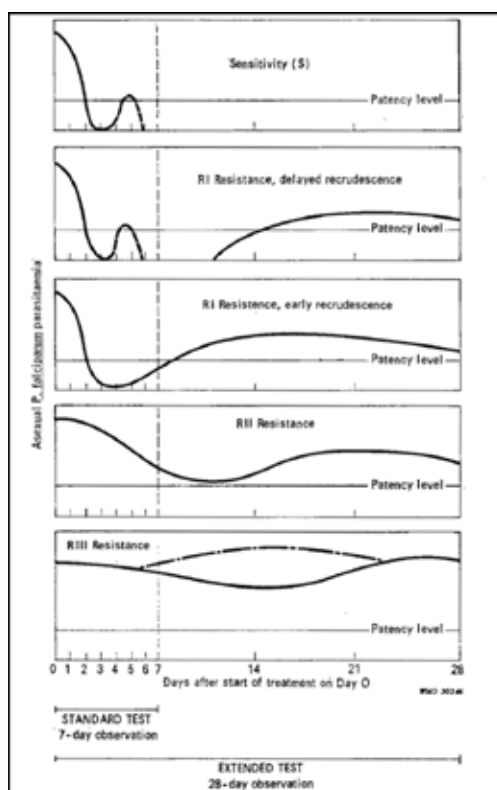


Figure 2.16. A WHO field test for response of malaria parasites to chloroquine (From World Health Organization. Chemotherapy of malaria and resistance to antimalarials: report of a WHO scientific group. Technical report series no. 329. Geneva: World Health Organization; 1973.)

shows an initial reduction in parasitemia after treatment but there is failure to clear the blood of asexual parasites and soon after an increase of parasitemia follows. RIII is the severest form of resistance in which parasitemia will either show no significant change with treatment or will eventually increase.

MDR malaria is considered when treatment failure occurs with three or more antimalarial agents. In this case, a combination of artesunate has been combined with mefloquine and is now the first-line regimen for MDR malaria in some Southeast Asian countries.

Classification of response to malaria treatment can be divided into early treatment failure, late treatment failure, and adequate clinical and parasitological response. Early treatment failure (ETF) is present when there is: (a) development of danger signs or severe malaria on Day 1, 2, or 3 in the presence of parasitemia; (b) parasitemia on Day 2 higher than the Day 0 count irrespective of axillary temperature; (c) parasitemia on Day 3 with axillary temperature of 37.5°C; and (d) parasitemia on Day 3 which is 25% of count on Day 0. This classification of ETF holds for both intense transmission and low to moderate transmission areas. Late treatment failure (LTF) is further divided into late clinical failure and late parasitological failure. The definitions for these two would differ depending on whether the area is an intense transmission area or a low to moderate one. Late clinical failure for intense transmission areas is defined as: (a) development of danger signs or severe malaria after Day 3 in the presence of parasitemia, without previously meeting any of the criteria for ETF; and (b) presence of parasitemia and axillary temperature equal to or greater than 37.5°C on any day from Day 4 to Day 14, without previously meeting any of the criteria for ETF. For low to moderate transmission areas, late clinical failure is defined as: (a) development of danger signs or severe malaria after Day 3 in the presence of parasitemia,

without previously meeting any of the criteria for ETF; and (b) presence of parasitemia and an axillary temperature of 37.5°C (or history of fever) on any day from Day 4 to Day 28, without previously meeting any of the criteria for ETF. Late parasitological failure for intense transmission areas is defined as presence of parasitemia on Day 14 and axillary temperature of 37.5°C without previously meeting any of the criteria for ETF or late clinical failure.

For low to moderate transmission areas, late parasitological failure is defined as presence of parasitemia on any day from Day 7 to Day 28 and axillary temperature of 37.5°C without previously meeting any of the criteria for ETF or late clinical feature. Adequate clinical and parasitologic response for intense transmission areas is defined as absence of parasitemia on Day 14 (Day 28 for low to moderate transmission areas) irrespective of axillary temperature without previously meeting any of the criteria of ETF, late clinical failure, or late parasitological failure.

In cases of renal failure in severe malaria, dopamine may be given at 3 to 5 µg/kg/minute. If the patient remains unresponsive despite adequate rehydration and other forms of therapeutic management, and blood urea and creatinine are rising progressively, dialysis is indicated.

For control of seizures, diazepam may be given at 10 mg intravenous (pediatric dose at 0.3 mg/kg IV up to 10 mg) or in cases of status epilepticus, phenytoin at a loading dose of 13 to 18 mg/kg and a maintenance dose of 3 to 5 mg/kg per day (pediatric dose: loading dose of 15-20 mg/kg slow IV push and maintenance dose of 5 mg/kg in two divided doses). The following are now not considered useful and are therefore not recommended in the management of cerebral malaria: corticosteroids, other anti-inflammatory agents, low molecular weight dextran, epinephrine, and heparin.

Proper management of malaria also includes general and supportive measures

especially in *P. falciparum* infections. If fluid replacement or blood transfusion is necessary, it must be administered with care to avoid pulmonary edema. Antipyretics and sponging for high fever are important especially in children to prevent convulsions. Blood sugar should be monitored regularly especially in severe malaria. If hypoglycemia develops, 50 mL of 50% dextrose (1.0 mL/kg for children) diluted in an equal volume of infusion fluid should be infused over a 5-minute period, followed by a continuous intravenous infusion of 5 to 10% dextrose.

Epidemiology

Malaria is the world's most important tropical parasitic disease. The disease kills more people than any other communicable disease except tuberculosis. In many developing countries, especially in Africa, malaria has an enormous toll on lives, medical costs, and days of labor lost. The geographical areas affected by

malaria have shrunk considerably over the past 50 years, but control is becoming more difficult, and past gains have been threatened. The spread of the disease is linked to activities like road building, mining, logging, and new agricultural and irrigation projects, particularly in "frontier" areas (e.g., forest fringe, mountain valleys and reclaimed areas). Elsewhere, disintegration of health services, armed conflict, and mass movements of refugees have worsened the malaria situation.

Malaria remains a public health problem today in more than 90 countries inhabited by a total of some 3.3 billion people (Figures 2.17 to 2.19). Of these, 2.1 billion are at low risk (<1 case per 1,000 population), 94% of whom live in areas outside the WHO African region. The 1.2 billion at high risk (>1 case per 1,000 population) live in the WHO African (47%) and Southeast Asian Regions (37%).

In 2010, the WHO reported an estimated 216 million cases of malaria, of which 81% or

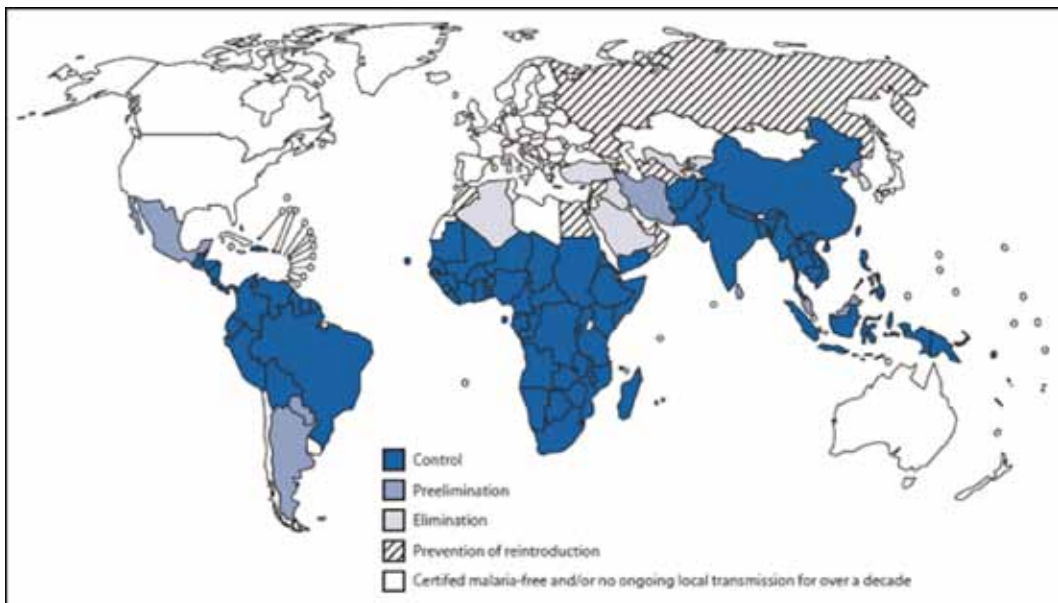


Figure 2.17. Global distribution of malaria: malaria-free and malaria-endemic countries in phases of control, elimination and prevention of reintroduction (From World Health Organization. World Malaria Report 2009. Geneva: World Health Organization; 2009.)

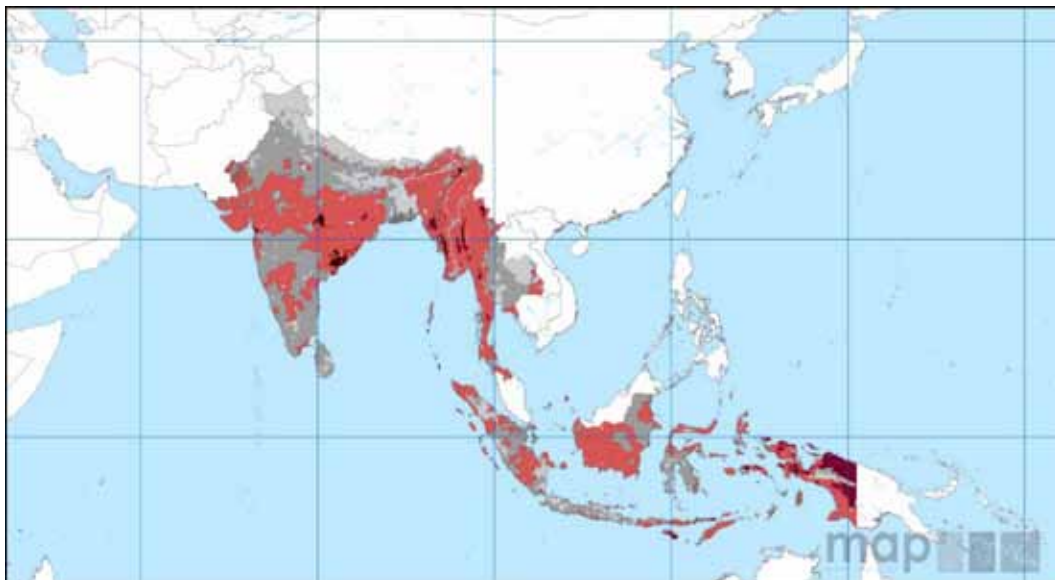


Figure 2.18. Distribution of malaria in the WHO Southeast Asia Region: areas in red indicate malaria-endemic countries (Accessed from <http://www.map.ox.ac.uk>)



Figure 2.19. Distribution of malaria in the WHO Western Pacific Region: areas in red indicate malaria-endemic countries (Accessed from <http://www.map.ox.ac.uk>)

171 million cases where in the African region, with the Southeast Asian Region accounting for another 13%. An estimated 655,000 malaria

deaths occurred in 2010, 91% of which were in Africa, and approximately 86% of these deaths were children under 5 years of age. The estimated incidence of malaria has fallen by 17% globally between 2000 and 2010. Large percentage reductions were seen in the European (99.5%), American (60%), and Western Pacific (86%) WHO regions. Likewise, malaria specific mortality rates have fallen by 25% between 2000 and 2010.

According to the World Malaria Report 2011, the WHO cites a decreasing number of malaria cases in a majority of countries belonging to the Western Pacific Region. A greater than 50% decrease in cases were reported for China, Philippines, Republic of Korea, Solomon Islands, and Vietnam, while a 25-50% decrease in the number of cases were seen in Lao People's Democratic Republic, Malaysia, and Vanuatu. No notable change in the number of malaria cases were seen in Cambodia and Papua New Guinea.

In the Philippines, malaria has not been included among the 10 leading causes of

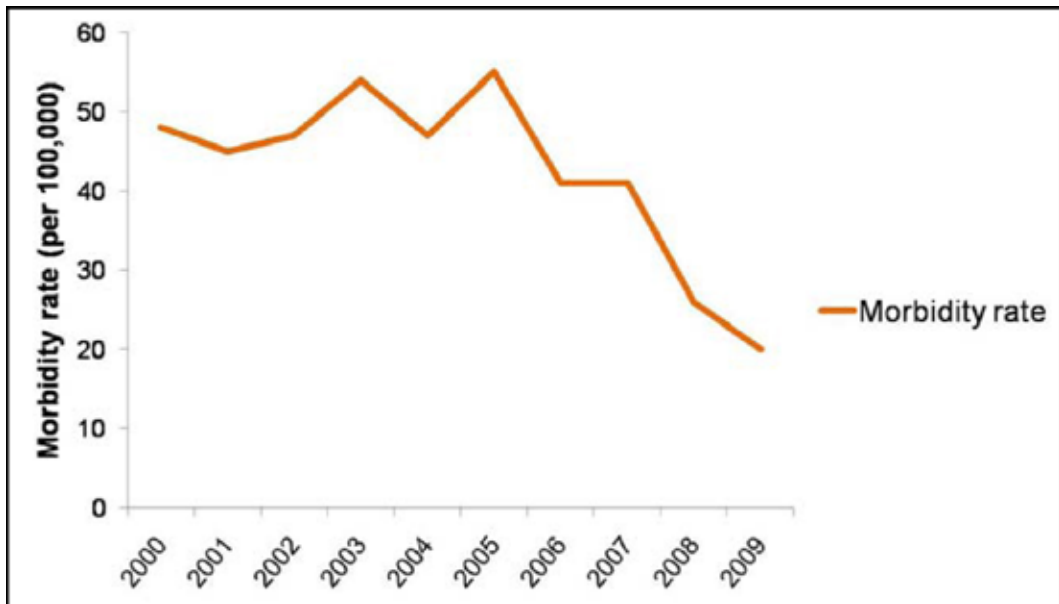


Figure 2.20. Malaria cases per 100,000 population in the Philippines from 2000 to 2009 (From Department of Health-National Center of Disease Prevention and Control. Malaria medium term development plan 2011-2016. Manila (Philippines): Department of Health; 2011.)

morbidity since 2006. Cases have been notably decreasing as reported in 2009 (Figure 2.20). However, disease prevalence, seen in the 2010 DOH-Malaria Control Program (MCP) report, remains markedly high in Regions IV-B, Caraga, III, XII, and II. There remains an estimated 10.8 million people still at risk for the disease, consisting mostly of farmers, indigenous cultural groups, miners, forest product gatherers, and soldiers. Fifty nine out of the 80 provinces in country are endemic for the disease, with 60.4% of the endemic provinces located in Luzon, 39.5% in Mindanao, and 0.1% in Visayas. As of 2009, the provinces of Cagayan, Isabela, Palawan, Sulu, and Tawi-Tawi comprise the five provinces having the highest number of malaria cases reported.

It appears that in areas of low malaria endemicity, there is a clustering of cases, resulting in pockets of high endemicity. Mortality rate for malaria has markedly decreased by 88.2% from 2005 figures to values

reported in 2009 (Figure 2.21). Similarly, the malaria morbidity rate has decreased by 58.3%, with 18,781 malaria cases reported in 2009. Among blood smears examined from 2005 to 2009, 69.4 to 80% of patients were diagnosed with *P. falciparum*, 17.0 to 23.4% with *P. vivax*, approximately 1% with *P. malariae*, and a small number of cases (0.5%) were diagnosed to have mixed malaria infection, falciparum and vivax malaria being the usual mixed infection.

Macrostratification of malaria endemic areas serves to classify the different provinces based on annual incidence of the disease in each respective province (Table 2.8). Macrostratification provides an opportunity for planning, policy making, and resource allocation of the provincial MCP. The number of provinces in Category A has been reduced from 26 in 2000, to nine in 2005 and finally to five in 2008. The values reported for Category B provinces have changed from 22 in 2000 to 31 in 2005, and to 27 in 2008. Malaria-free

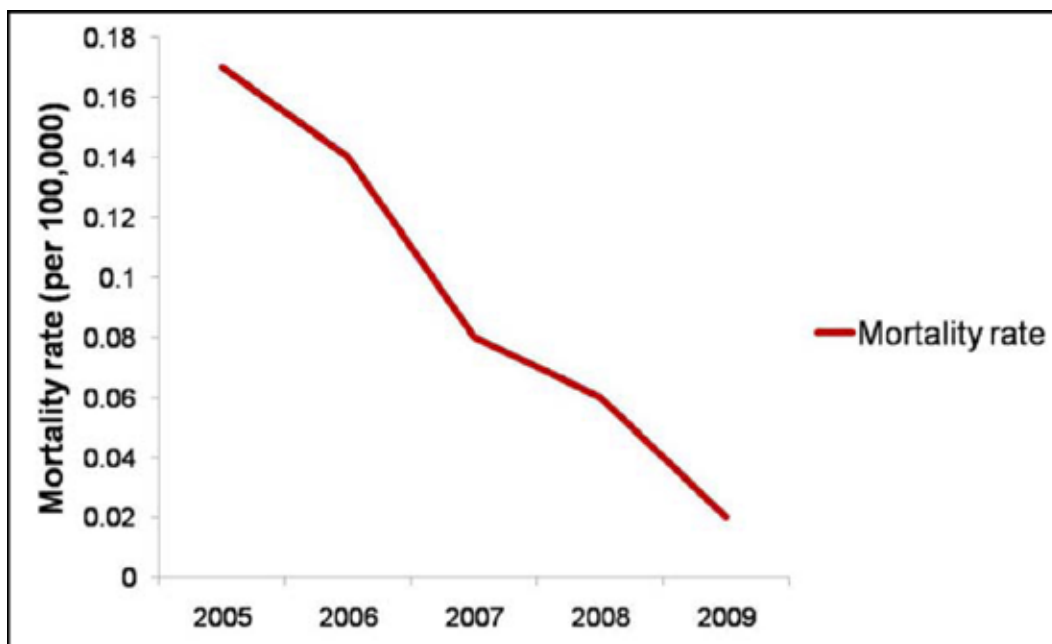


Figure 2.21. Malaria-related deaths per 100,000 population in the Philippines from 2005 to 2009 (From Department of Health-National Center of Disease Prevention and Control. Malaria medium term development plan 2011-2016. Manila (Philippines): Department of Health; 2011.)

Table 2.8. Macrostratification of malaria endemic provinces according to annual incidence of malaria

Category	Annual incidence of malaria
A	≥1000 cases
B	100 to <1000 cases
C	<100 cases
D	No documented indigenous case for the past 5 years
Source: Department of Health. Administrative Order no. 14 series of 1996: Technical guidelines on stratification of areas. 1996.	

provinces have increased from 13 in 2000, to 22 in 2009. Four provinces in Category A, eight provinces in Category B and eight provinces in Category C have been reclassified to the immediate lower categories respectively, from 2005 to 2009. Nueva Ecija is noted to have shifted into a higher category (Figure 2.22).

Peak transmission occurs at the beginning and at the end of the rainy season. In the

Philippines, the principal malaria vector is *Anopheles minimus* var. *flavirostris*, a night biter, which prefers to breed in slow flowing, partly shaded streams that abound in the foothill areas. Occasionally, it has the ability to adapt to or utilize new habitats such as irrigation ditches, rice fields, pools, and wells. In Palawan, it was observed to be mildly exophagic and zoophilic. Its horizontal flight range has been reported to be about 1 to 2 km. *Anopheles litoralis* is associated with malaria transmission in the coastal areas of Mindanao, particularly in Sulu. *Anopheles maculatus* coexists with *A. flavirostris* in the portion of streams exposed to sunlight. They appear to be responsible for malaria transmission at higher altitudes. *Anopheles mangyanus* has the same breeding habitats and seasonal prevalence as *A. flavirostris* but appears to prefer habitats located in forest fringe.

Malaria can also be transmitted through blood transfusion from infected donors, and

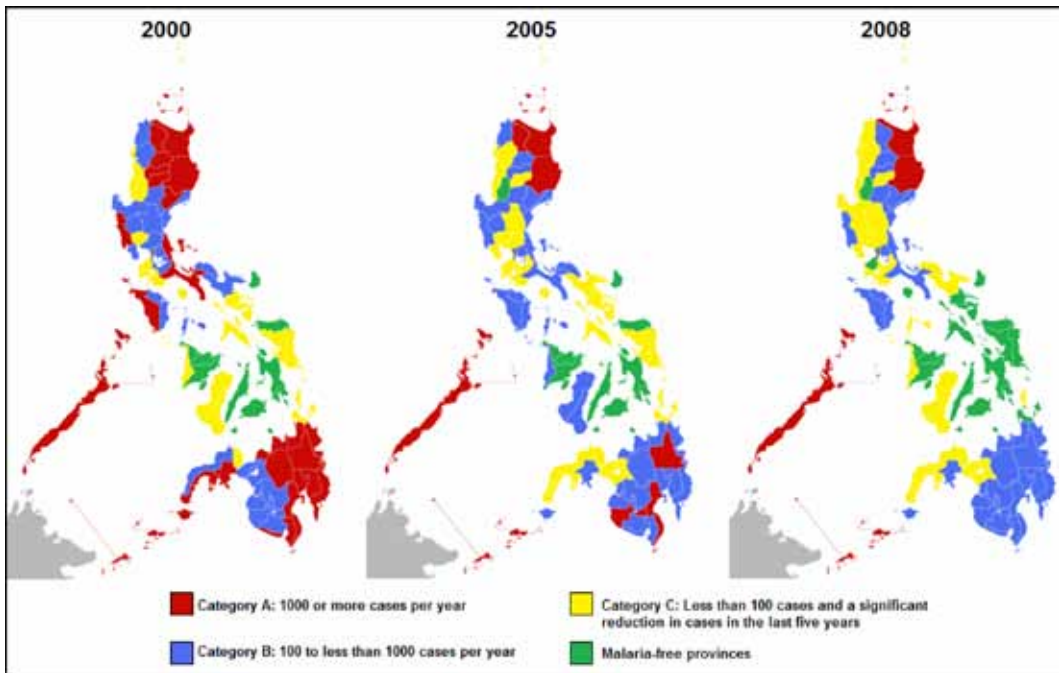


Figure 2.22. Macrostratification of provinces in the Philippines according to category by average malaria cases: note that the Isabela, Cagayan, Palawan, Sulu, and Tawi-Tawi remain Category A provinces as of 2008 (From Department of Health-National Center for Disease Prevention and Control. Malaria control program. 2009.)

by contaminated needles and syringes. Blood from semi-immune donors without clinical symptoms may contain malaria parasites. In congenital malaria, infected mothers transmit parasites to their child before or during birth.

The evaluation of the amount and conditions of transmission of malaria in a given locality is called the malaria survey. Disease control efforts must always take into consideration the findings of the malaria survey. The survey involves looking into epidemiologic data regarding the disease, such as malaria mortality and morbidity, investigations relating to the human host, and investigations relating to the insect vector. Investigations relating to the human host include blood and spleen examinations. Investigations relating to the vector, on the other hand, may include identification of mosquito vectors and their

breeding sites, and estimation of mosquito density.

Prevention and Control

Early diagnosis and prompt treatment of malaria are essential for malaria control. Breeding sites of *Anopheles flavirostris* should be detected early and contained. Personal protection measures against mosquito bites are also helpful and cost-effective. The use of insecticide- (permethrin or deltamethrin) treated nets (ITNs) and long lasting insecticide-treated nets (LLIN) remains the major vector control strategy coupled with indoor residual spraying (IRS), with the latter used in epidemic situations, areas with stable transmission but without reduction of malaria incidence, and areas of displaced populations. Wearing of light colored clothing, which cover most of the

Table 2.9. Treatment of malaria infection

Common name	Chemical class	Clinical use	Resistance
Artemisinins (artemether, artesunate, dihydroartemisinin)	Sesquiterpine lactone endoperoxide	In artemisinin-based combination therapies (ACTs)	Possibly emerging
Lumefantrine	Arylamino alcohol	Most common first line anti-malarial therapy in Africa, in combination with artemether Artemether plus lumefantrine (AL) – most common drug combination used in uncomplicated falciparum malaria	No evidence of high level resistance
Amodiaquine	4-aminoquinoline	In combination with artesunate in parts of Africa	Limited cross resistance with chloroquine
Piperaquine	Bisquinoline	In combination with dihydroartemisinin in parts of Southeast Asia	Observed in China following single drug therapy
Mefloquine	4-methanolquinoline	In combination with artesunate in parts of Southeast Asia	Prevalent in Southeast Asia
Pyronaridine	Acridine type Mannich base	Being registered for combined use with artesunate	No cross-resistance with other drugs
Quinine/quinidine	4-methanolquinoline	Mainly used for the treatment of severe malaria, often in combination with other antibiotics Drug of choice in severe malaria	Exists at a low level
Atovaquone	Naphthoquinone	In combination with proguanil (a biguanide) for treatment or prevention	Has been observed clinically
Chloroquine	4-aminoquinoline	Former first line treatment, together with sulfadoxine-pyrimethamine (SP) of uncomplicated falciparum malaria Remains drug of choice for vivax malaria	Widespread
Pyrimethamine	Diaminopyrimidine	For intermittent preventive treatment, combined with sulfadoxine (a sulfonamide)	Widespread
Primaquine	8-aminoquinoline	For eliminating liver-stage parasites, including dormant forms of <i>P. vivax</i> Drug of choice for gametocytes and hypnozoites	Unknown
Source: Fidock DA. Drug discovery: priming the antimalarial pipeline. Nature. 2010;465: 297-298.			

body (since dark colors attract mosquitoes), using insect repellants containing DEET (N,N-diethyl-m-toluamide, optimally as a 35% concentration lotion) on exposed parts of the body, using a insect spray containing pyrethrum in living areas, and use of permethrin insecticide as a repellant spray for clothing have known to be effective personal protection measures as well.

Chemoprophylaxis may be protective to travelers who have no immunity to malaria, although no chemoprophylactic regimen ensures 100% protection. Because of this, precautions to avoid mosquito bites are needed even if antimalarials have been taken. Prophylactic drugs should be taken with good compliance for the duration of the stay and should be continued four weeks after the last possible exposure to infection since the parasites may still emerge from the liver after this period. An exception would be atovaquone/proguanil which can be stopped one week after return. Chloroquine is only recommended for areas where malaria is exclusively due to *P. vivax* or where there is low risk of chloroquine-resistant *P. falciparum*. Those traveling to areas where levels of resistance to chloroquine are high may use mefloquine, doxycycline or atovaquone/proguanil. Strategies implemented to control malaria in pregnancy include the use of ITNs and intermittent preventive treatment, which involves providing all pregnant women with at least two preventive treatments of an effective antimalarial drug.

Work is on-going for the development of an effective malaria vaccine. Among the vaccine types being developed are the “sporozoite” vaccines, “asexual” vaccines, and the “altruistic” or “transmission blocking” vaccines. Combination vaccines derived from multiple parasite life stages are also being developed.

Malaria control also includes proper vector control. This can be done through environmental modification, biological control which includes using larvivorous fish in streams

and rice fields and bacterial insecticide (PG-14 *Bacillus thuringiensis*), and chemical control such as the use of mosquito repellants and insecticide treated mosquito nets.

In the field of molecular entomology, stable germline transformation of the *Anopheles* mosquito is being investigated. This research involves inserting genes (e.g., immune response genes) that will inhibit the development of the parasite in the mosquito. With the recent sequencing of the genomes of *Plasmodium falciparum* and of the *Anopheles* mosquito, new areas of research for malaria treatment and prevention are now being explored.

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Babesia spp.

Florencia G. Claveria

Babesia spp. is a hemosporidian parasite that causes babesiosis, a hemolytic disease commonly referred to as tick, splenic, redwater, Texas, or Nantucket fever. *Babesia* was first documented in cattle in 1888 by Dr. Victor Babes, a Romanian scientist, who described symptoms of severe enzootic hemoglobinuria. *Babesia* has a worldwide distribution and comprises approximately 73 to 100 species infecting wild and domestic animals, as well as humans. *Babesia* spp. are generally specific to their vertebrate hosts, and are transmitted by Ixodidae or hard ticks. In humans, transmission through blood transfusion, organ transplantation, and transplacental route have been documented.

As babesiosis affects a wide range of domestic animals, it undeniably brings about huge economic losses to agriculture. The increasing association between man and animals has resulted in increased infection, not only among the immunocompromised individuals but also among the general populace. To increase public health awareness, a better understanding of the parasite biology and its tick vector, the disease it causes, and its epidemiology particularly in the Asia-Pacific, is imperative.

Parasite Biology

Babesia is a heteroxenous parasite requiring mammals as primary hosts, and ticks as intermediate hosts or vectors. On account of the disparity in the morphological features of the intra-erythrocytic forms in different host species, there exist about 100 species or forms. The tendency of *Babesia* spp. to take on pleomorphic forms in different hosts obscure their identification at the species level. For example, its close relative, the *Theileria* spp.

that infect red blood cells (RBCs) are generally smaller and can easily be misidentified. *Babesia* spp. are largely host-specific, and non-host specific species utilize only a narrow range of mammalian hosts like cattle and rodents. Some species infecting mammals exhibit cross-infectivity and induce cross-immunity between host species.

Hard ticks (family Ixodidae) are the known biological hosts of *Babesia*, however, transmission of *Babesia* by the soft tick, *Ornithodoros erraticus* has been reported. Tick species that have been established as putative vectors are *Boophilus* spp., *Rhipicephalus* spp., *Ixodes* spp., *Hyalomma* spp., *Haemaphysalis* spp., and *Dermacentor* spp. Of the 53 tick species incriminated as vectors, only 17 have been recognized. The transmission to humans of the rodent *B. microti* and the cattle forms of *Babesia* is generally associated with *Ixodes* spp.

Unlike the genus *Plasmodium* and *Theileria*, *Babesia* does not undergo exo-erythrocytic merogony; daughter progeny are not housed in parasitophorous vacuoles; and residual bodies are usually non-existent in infected RBCs.

The *Babesia* life cycle undergoes three developmental phases. In the mammalian host, (a) merogony in the RBC; and in the tick host, (b) stages of gamogony in the gut and epithelium; and, (c) sporogony accompanied with multiple fission in various cells and organs forming sporokinets, and the development of infective sporozoites. A few hours after blood ingestion, the intra-erythrocytic merozoites in the gut of engorged ticks undergo morphologic, physiologic/metabolic, and antigenic changes, and differentiate into gametocytes that eventually develop into gametes. Post-fertilization, the zygote begins to infect the gut epithelial cells where it undergoes multiple

fission, and eventually forms sporokinetes. Once the sporokinetes are released, they continue to infect and multiply in various organs, including the ovaries of the replete tick, until death ensues. The transovarian route represents one pattern of parasite transmission in the vector, which terminates with the death of the vector.

With the passage of sporokinetes to eggs (transovarian), similar cycles of multiple fissions continue to take place in the embryo and in

the organs of the larva, nymphs, and adult ticks. With the stage-to-stage (transstadial) transmission, each of the developmental stages is generally capable of parasite transmission to mammals. The complicated phase of *Babesia* life cycle in the tick vector ends with the formation of the infective sporozoites in various organs or in the salivary glands, for subsequent transmission to the mammalian hosts during blood feeding (Figure 2.23).

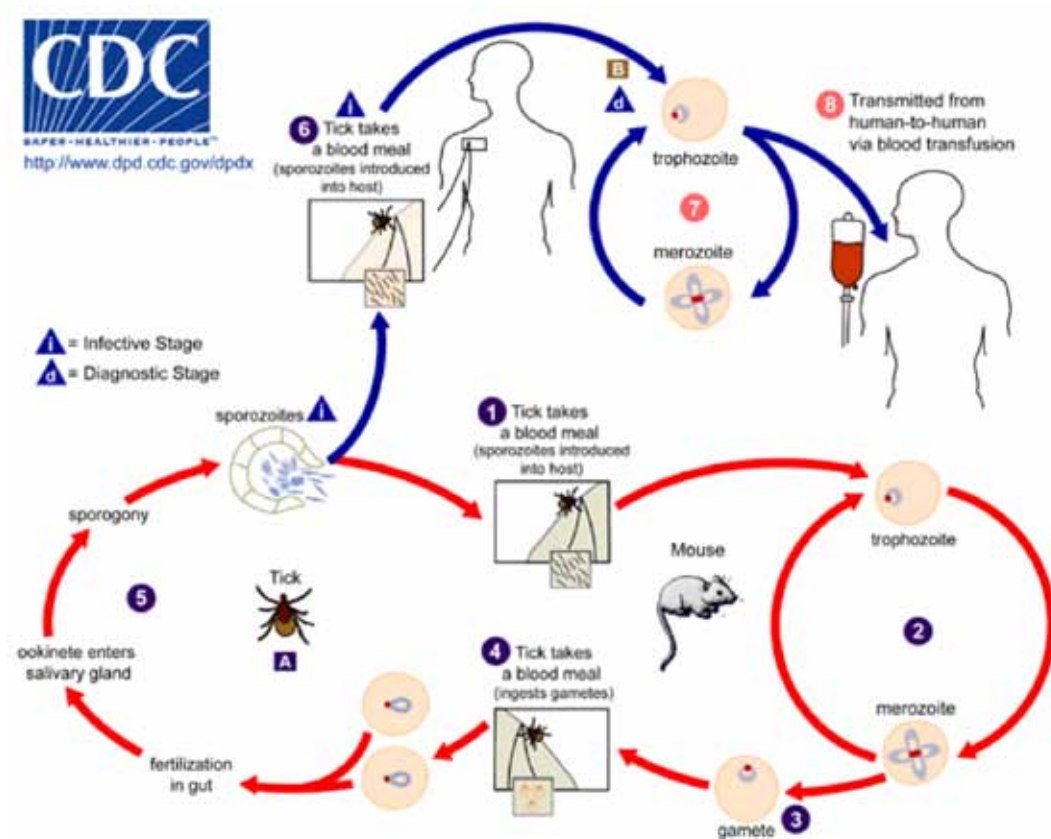


Figure 2.23. Life cycle of *Babesia* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

Pathogenesis and Clinical Manifestations

Smaller forms like *Babesia bovis* (2.4 μm by 1.5 μm) and *B. equi* (2 μm by 1 μm) are more pathogenic, while larger forms such as *B.*

bigemina (4-5 μm by 2-3 μm) and *B. caballi* (3 μm by 2 μm) are less virulent. Several factors can influence the susceptibility of hosts to infection, like the age and breed of farm animals, and the health and immune state of humans.

Both innate and acquired immunity contribute to the resolution of the primary infection and provide protection against subsequent exposures. The importance of the spleen in the elimination of both the parasites and infected RBCs is seen in the increased susceptibility of splenectomized or inherently asplenic mice to infection. Murine hosts depleted of macrophages exhibit either high mortality or become unprotected when exposed. The transfer of primed macrophages, as opposed to transfer of primed T-cells, provides protection against *B. microti* in naive mice. The resolution of babesiosis is principally mediated by gamma interferon produced by CD4⁺ T helper-1 cells, alongside macrophage activation. While B cell-depleted mice are capable of controlling primary infection, antibodies are still useful in the clearance of extracellular parasites in circulation. Despite low parasitemia in the peripheral circulation among infected cattle, infected erythrocytes are sequestered in the capillary beds causing cerebral babesiosis, a similar manifestation of falciparum malaria.

In humans, infections with *B. microti* or *B. microti*-like species may be asymptomatic or may result in mild to severe clinical signs and symptoms. Fatigue, malaise, anorexia, and weight loss begin to manifest approximately one to six weeks post-exposure followed by non-periodic or intermittent fever (38–40°C), chills, and sweats accompanied with headache, myalgia, arthralgia, nausea, vomiting, and prostration. The patient may also manifest emotional lability, depression, and hyperesthesia. In severe cases, hemolytic anemia and hemoglobinuria with jaundice become apparent, with pulmonary edema being the most frequently observed complication of the disease. The severity of infection with possible fatal outcome is generally associated with the elderly, the splenectomized and immunocompromised, and those manifesting evidence of Lyme disease.

Diagnosis

Babesia parasites are usually detectable in blood smears only during the acute stage of the infection, and animals that survive the initial infection can become lifelong carriers. Previous infections can be demonstrated serologically.

Definitive diagnosis requires direct microscopic examination of Giemsa-stained peripheral blood smears for the presence of *Babesia*, showing its established unique morphological features. To rule out the misdiagnosis from closely related hemosporidians like *Plasmodium* spp., and the causative agents of Lyme disease, and granulocytic ehrlichiosis infecting humans, the parasite dimensions and pleomorphism (ring form, pear-shaped, and “Maltese cross” or tetrad form) need to be ascertained, including the absence of pigments in infected RBCs. In cases of low-grade infection or parasitemia, detection can be very difficult, thus, appropriate serological assays, molecular gene analyses, and epidemiologic data, including data on ticks and reservoir or carrier hosts (epizootiological), may be extremely useful.

Several serological tests are generally employed for the detection of babesiosis. The immunofluorescent assay (IFA) is widely used in acute cases and in epidemiological studies. Although sufficiently sensitive, IFA has the following drawbacks: non-differentiation between active and past exposures, possibility of cross-reactivity between antigens of closely related species, and subjectivity in the quantification of the intensity of fluorescence. At lower dilutions and during the acute phase, anti-*B. microti* antibodies cross-react with antigens of other *Babesia* spp. Also, antigens of *B. microti* occasionally cross-react with sera of confirmed malarial cases with a >1:16 antibody titer. To rule out possible cross-reactions, a 1:64 serum dilution is highly recommended. In cases of low parasitemia, experimental inoculation of specific pathogen-free hamsters with infected

blood or NOD/sch-*scid* mice with the patient's blood can be useful in parasite detection and identification.

The polymerase chain reaction (PCR) is highly specific and is generally considered to be the gold standard for *Babesia* detection. It is, however, unrealistic for epidemiologic surveys because it is time consuming and expensive. The current practice is the use of PCR in the isolation of the SSU rDNA from asymptomatic patients, followed by gene sequencing and its comparison with known SSU rDNA gene sequences from pathogenic strains.

The continued work on the isolation of specific and highly immunogenic antigens of *Babesia* species and isolates, and their intended utilization in the development of immunochromatographic test (ICT) is practicable for epidemiologic surveys in the field. ICT is simple, quick, reliable, sensitive, and inexpensive, and has gained acceptability in the diagnosis of both acute and latent infections. Currently, there are ICT strips or dipsticks employed in the detection of infected livestock.

Treatment

The standard treatment of human babesiosis utilizes a drug combination of clindamycin and quinine, or azithromycin and atovaquone. The clindamycin and oral quinine combination was first used in 1982 in a newborn infant suffering from babesiosis, and since then, this combination has become the drug of choice. Clindamycin is given 1.2 g intravenously twice a day or 600 mg orally three times a day, and combined with oral quinine at a dose of 650 mg three times a day. Clindamycin-atovaquone combination is efficacious in clearing the parasites in normal individuals and prevents recurrence of infection, but produces adverse effects like vertigo, tinnitus, and gastrointestinal symptoms. Supportive and symptomatic management is important. In severe cases where there is progressive exacerbation of hemolytic anemia, total blood exchange may be indicated.

The drug combination azithromycin and atovaquone is as effective as clindamycin-quinine, with less adverse effects. Both drug combinations are ineffective in suppressing disease progression in immunosuppressed patients. Very recently, there have been reports of immunocompromised patients who, during 28 days of uninterrupted treatment with azithromycin-atovaquone, manifested recurrence of marked parasitemia, suggesting the development of drug-resistant *B. microti*.

Artemisinin, pyrimethamine, and pamaquine can strongly inhibit the growth of *B. equi* and *B. caballi* *in vitro*. Pyrimethamine can indirectly interrupt the parasite life cycle through its inhibitory effect on dihydrofolate reductase, essential in folate metabolism, while pamaquine can interfere in the recycling of endosomal proteins into the plasma membrane by direct interaction with the endosomes.

Epidemiology

Babesiosis is essentially a zoonotic infection, regarded of major economic importance to livestock, particularly in the cattle and horse industry. Its documentation in humans worldwide has increased its recognition as a disease of public health concern. The first human case attributed to the cattle form was reported in 1956, in Europe, and followed with reports of more cases in Europe and in North America, including the discovery of the transmission of a *B. microti*-like species to humans in 1970. A review of the 136 human cases examined in New York (1982-1991) revealed almost all cases were among those living in Suffolk, Long Island. Of the 103 (76%) who were hospitalized, seven patients previously underwent splenectomy, 31 patients had symptoms of babesiosis and Lyme disease, and seven died. The Asian cases reported have been few and sporadic with the first records in 1984, in Yunnan, China, and a recent report in Japan attributed to *B. microti* (Table 2.10).

Table 2.10. Summary of human cases of babesiosis reported in some Asian countries

Location	Signs and Symptoms	Diagnosis
Yunnan, China	Fever, jaundice, anemia, cutaneous edema; myalgia, malaise, nausea, prolonged and repeated illness; periodic fever (39.5-41.0°C), with renal transplantation prior to onset of fever	Initially malaria, then babesiosis
Taiwan	Headache, malaise, fatigue, dull pain in upper abdomen, and frequent mild fever, chill for a few months, hemolytic anemia	Gallstone with hepatosplenomegaly and babesiosis
Mongolia	High fever, chill, nightly sweating, myalgia, low grade parasitemia	Babesiosis
Japan	Fever, malaise, excretion of dark-colored urine, progressive hemolytic anemia	Babesiosis

The Centers for Disease Control and Prevention, USA has confirmed more than 40 human cases that contracted the disease from transfusion of packed RBC and tested positive for anti-*B. microti* antibodies. In Asia, the two cases have been associated with renal transplantation and blood transfusion. Thus, subclinical or asymptomatic cases cannot simply be ignored, considering their potential role in the spread of human babesiosis.

Phylogenetic analyses of the gene sequences of the SSU rDNA of *Babesia* spp. obtained from human cases helped clarify three patterns or groupings, worldwide, namely: (a) human babesiosis attributed to the *B. divergens*-related parasites in Europe; (b) human babesiosis caused by *B. microti* principally in the Northeastern USA; and (c) human babesiosis caused by newly emerging species, the WA1-type in the Western USA, tentatively grouped with *B. microti* or alternatively with *Theileria* spp. Recently in Italy and Austria, parasites obtained from splenic cases revealed SSU rDNA sequences more closely related to *B. odocoileus*, a species that bears morphological, molecular, and immunological similarities with *B. divergens*. The *B. divergens*-related species now has been expanded to include *B. odocoileus* and possibly *B. bovis*. In Asia, the etiologic agent of human babesiosis has been identified as *B. microti* or *B. microti*-like isolate or strain.

Human cases recorded in China were generally among farmers living in close

habitation with livestock and wild animals, and where ticks were abundant. The parasites detected were pyriform-shaped, suggestive of *Babesia*. One case recorded in the rural area in Southwestern Taiwan was serologically and morphologically diagnosed with a chronic and subclinical infection of a geographic isolate of *Babesia* named Taiwan isolate (TW1). The detection of anti-*Babesia* antibodies in 83% *Rattus coxinga* endemic in the locality where the Taiwanese patient lived, suggested the rodents as the highly likely source of infection. The SSU rDNA isolated from the Japanese patient and from the NOD/sch-*scid* mice inoculated with the patient's blood revealed 99.2% sequence homology with the US *B. microti* SSU rDNA (Genbank/EMBL/DBJ: U09833).

In Japan the wild rodents, *Apodemus speciosus* and *A. argenteus*, are infected with *B. microti*-like forms. In Taiwan, the bandicoot rats, *Bandicola indica*, and the spiny rat, *R. coxinga*, carry morphologically similar *B. microti*-like forms. The TW1 isolated from the first human case in Taiwan is serologically related to the US *B. microti* SSU rDNA.

In the Philippines, studies on animal babesiosis have been limited and mainly concentrated on hematological parameters and clinical manifestations in cattle *B. bigemina* and *B. argentina* (syn. *B. bovis*), and *B. canis*. Using the ICT, 13 (28%) stray dogs in an impounding facility in Dasmarinas, Cavite tested positive for anti-p50 truncated *B. gibsoni* antigen. The dogs

had infestation mainly with *Rhipicephalus* ticks, suggestive of their putative role in the spread of canine babesiosis in the country.

Slaughtered and race horses in the Philippines tested seropositive for *B. caballi* and/or *B. equi* infection, using ICT containing a recombinant *B. caballi* 48-kDa rhoptry protein (rBc48) and a recombinant truncated *B. equi* merozoite antigen 2 (rEMA-2t). Serological data correlated well with the detection of the morphologies of the specific etiologic agent(s) in blood smears.

In Europe and the USA, the *Ixodes ricinus* and *Ixodes trianguliceps*, and the *Ixodes scapularis* and *Ixodes pacificus* are the principal vectors, respectively. In Asia, the tick vectors are poorly established. The predominance of *Ixodes granulatus* in Southeast Asian countries makes it a favorable vector, though this warrants confirmation (Plate 2.17). In Japan, *Ixodes ovatus* has been suggested as a highly likely tick vector of human babesiosis for its human biting activity.



Plate 2.17. *Ixodes* sp. A. Non-engorged female. B. Engorged female. C. Mouthparts showing the hypostome (H), pedipalp (P), and scutum (S). (Courtesy of Ms. Mary Jane Cruz-Flores and Dr. Florencia Claveria)

Prevention and Control

Babesiosis is essentially a zoonotic infection, and its transmission to man through the bite of the tick vector is incidental. Effective prevention strategies include avoiding tick-infested areas, remaining covered with clothing, and immediately removing any attached ticks. As the parasite is capable of stage-to-stage passage, each of the developmental stages is capable of parasite transmission to mammals. The application of bug repellents in clothes like N,N-diethyl-meta-toluamide (DEET) and acaricides like permethrin may be useful. The rodent population should be controlled as well, as they are major carriers or reservoirs of the parasite.

In low grade and chronic infections, babesiosis is generally asymptomatic. Worldwide,

serological surveys reveal more of subclinical or asymptomatic cases. Human cases of babesiosis are generally associated with splenectomized and immunocompromised patients, and noteworthy are the cases acquired through blood transfusion and organ transplantation. There may be a need to consider the inclusion of screening procedures for *B. microti* for blood and organ donors in high risk areas.

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Blood and Tissue Flagellates

Vicente Y. Belizario, Jr., Julius R. Migriño, Jr.

Locally acquired infections due to the blood and tissue flagellates have not yet been documented in the Philippines. However, because of fast and easy travel, as well as increased human migration, imported cases from endemic countries may become future sources of local infection. This scenario is possible because the vectors of *Trypanosoma cruzi*, *Triatoma* and *Rhodnius* bugs, are found in the country. In the same manner, the Philippines has a number of *Phlebotomus* spp., which can serve as vectors for *Leishmania* spp.

Trypanosoma cruzi

Trypanosoma cruzi is the etiologic agent of Chagas disease or American trypanosomiasis. This is the only parasite that was discovered and studied before it was known to cause a disease. More than 100 years ago, Carlos Chagas found that the trypanosomes he dissected from the intestine of a triatomid bug were the same parasites found in the blood of a child suffering from fever and enlargement of the lymph nodes. Since then, the understanding behind the disease that this protozoan causes has shown profound changes in terms of pathophysiology, diagnosis, and treatment.

Parasite Biology

T. cruzi belongs to the trypanosome group Stercoraria. Trypanosomes under this group multiply within the mammalian host in a discontinuous manner. Unlike other trypanosomes, it is an intracellular parasite, with myocytes (particularly myocardial tissues) and cells of the reticuloendothelial system being the most heavily infected cells. Other tissues that

get infected include the skin, gonads, intestinal mucosa, and placenta.

It has been well established that the arthropod vector responsible for propagating this parasite are the reduviid bugs, belonging to the genera *Triatoma*, *Panstrongylus*, and *Rhodnius*. These arthropods thrive under squalid housing conditions such as thatched roofs and mud walls, commonly seen in poor rural communities. Zoonotic mammalian reservoir hosts have been identified, including domestic animals, armadillos, raccoons, rodents, marsupials, and even some primates.

T. cruzi exhibits all four stages of development: amastigote, promastigote, epimastigote, and trypomastigote. In humans, trypomastigotes are found in the bloodstream, and amastigotes in tissue cells (Figure 2.24). Inside its insect vector, the amastigote, epimastigote, and promastigote forms occur in the midgut, while the infective metacyclic trypomastigote appear in the hindgut.

Amastigotes are round or ovoid in shape and measure from 1.5 to 4 μm in diameter. They are usually found in small groups of cyst-like collections in tissues.

The long slender trypomastigotes are 16 to 20 μm in length while the short, stumpy forms measure around 15 μm (Plate 2.18). The posterior end is usually pointed. The undulating membrane is narrow with two to three undulations, and a single thread-like flagellum originating near the kinetoplast provides the parasite with mobility. In stained specimens, trypomastigotes are characteristically C-shaped. They have also been described as U- or S-shaped with a prominent kinetoplast, characteristic of the species.

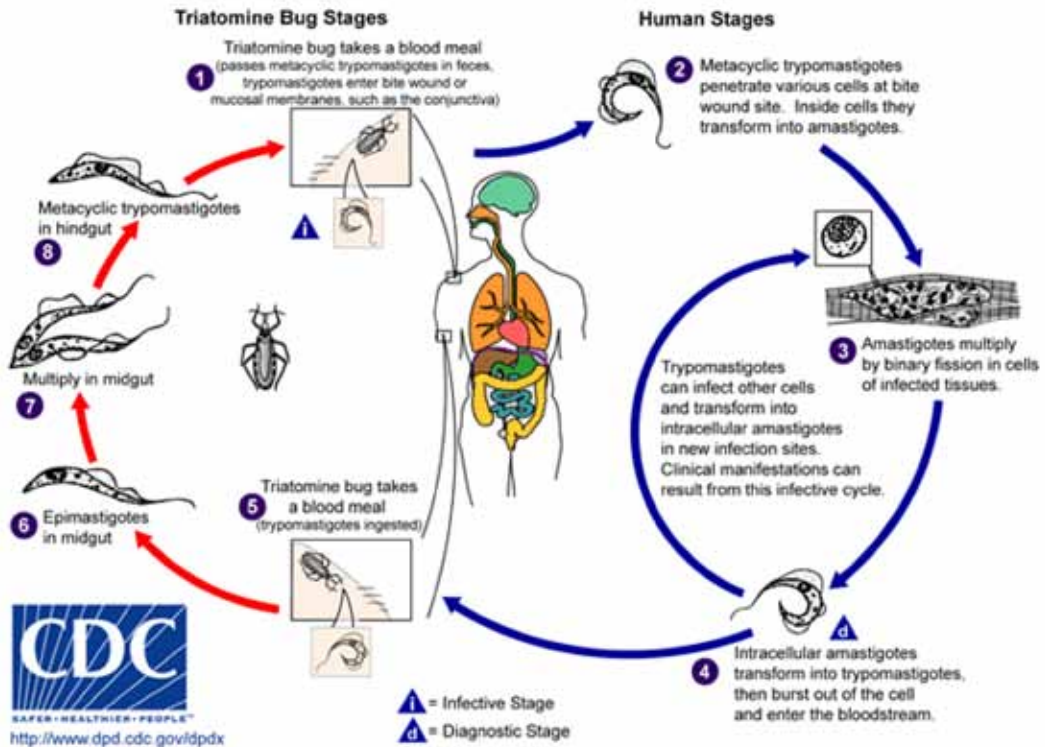


Figure 2.24. Life cycle of *Trypanosoma cruzi*
(Accessed from www.dpd.cdc.gov/dpdx)

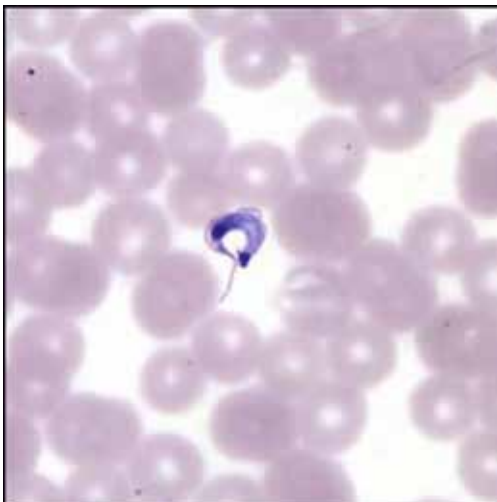


Plate 2.18. *Trypanosoma cruzi* trypomastigote in thin blood smears stained with Giemsa
(Accessed from www.dpd.cdc.gov/dpdx)

The trypomastigotes of *T. cruzi* do not multiply in the bloodstream. Soon after their entry into the human host, the metacyclic trypanosomes are engulfed by macrophages of the reticuloendothelial system and multiply through binary fission as amastigotes. Amastigotes develop into trypomastigotes, and the cells lyse in 4 to 5 days. The released trypomastigotes enter the bloodstream, ready to replicate again once they enter another cell or are ingested by an insect vector. Once ingested by the intermediate host, the trypomastigotes pass through the posterior portion of the insect's midgut and become epimastigotes, where they multiply via longitudinal fission. Infective metacyclic trypomastigotes appear in the insect's rectum 8 to 10 days after infection, and are passed in the bug's feces. The metacyclic trypomastigotes gain entry

into the body through broken skin, or through mucous membranes that are rubbed with fingers contaminated with the bug's feces.

Pathogenesis and Clinical Manifestations

Chagas disease can be divided into an acute and a chronic phase. The acute phase is characterized by a focal or diffuse inflammation mainly affecting the myocardium. Non-specific signs and symptoms, such as fever, malaise, nausea, vomiting, and generalized lymphadenopathy often accompany the acute phase. Cutaneous manifestations of the disease can sometimes appear during this phase, usually associated with a localized inflammatory reaction at or near the site of inoculation. Chagomas are furuncle-like lesions associated with induration, central edema, and regional lymphadenopathy. These lesions represent the site of entry of the parasite. If the parasite penetrates through the conjunctiva, eyelid swelling called Romaña's sign may form. This lesion is characterized by unilateral painless bupalpebral edema and conjunctivitis, and may involve the lacrimal gland and surrounding lymph nodes. After 1 or 2 months, symptoms resolve, and the patient goes into a latent or indeterminate, but usually asymptomatic phase. During this phase, patients infected with *T. cruzi* are still capable of transmitting it to others through insect vectors, blood transfusion, or organ transplantation.

The pathophysiology of the chronic phase of the disease was initially thought to be autoimmune in nature; however, this is controversial. Newer evidence shows that chronic Chagas disease is multifactorial, and dependent on the interaction between parasite and host. Nonetheless, the chronic phase is manifested by fibrotic reactions that cause injury to the myocardium, cardiac conduction network, and enteric nervous system (decrease in nerve ganglia leading to megasyndromes). The heart is the primary organ affected during this phase, which may

result in cardiomegaly, congestive heart failure, thromboembolism, and even arrhythmias. Less severe signs and symptoms associated with the chronic phase of the disease include chest pain, palpitations, dizziness, syncopal episodes, abnormal electrocardiogram findings, achalasia associated with megaesophagus, and chronic constipation associated with megacolon. About one-third of patients in the latent stage develop some manifestation of chronic Chagas disease after several years or decades. The majority of symptomatic, chronic patients manifest with the cardiac form, while the rest develop the gastrointestinal form.

Diagnosis

A complete patient history is the primary tool for diagnosing Chagas disease. Possible exposure to *T. cruzi* should be established, and risk factors such as place of residence or work, recent blood transfusion in an endemic area, and contact or exposure to *T. cruzi* intermediate host should be evaluated.

The definitive diagnosis of Chagas disease during its acute phase relies on direct visualization of the parasites in thick and thin blood smears using Giemsa stain. Cerebrospinal fluid (CSF), tissue samples, or lymph can also be used for parasite visualization. However, only in the first two months of acute disease can *T. cruzi* trypomastigotes be seen by direct examination. Other diagnostic techniques include concentration methods (microhematocrit), blood culture, and polymerase chain reaction (PCR). Xenodiagnosis, wherein laboratory-reared triatomine bugs are allowed to feed on suspected patients and are later examined for the presence of *T. cruzi* metacyclic trypomastigotes, may also be utilized as a diagnostic modality.

During the chronic phase, a variety of serologic tests may be used, such as enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination, indirect immunofluorescence, and PCR. The WHO recommends using at least two techniques with concurrent positive

results before a diagnosis of Chagas disease is made. For the cardiac form of the disease, ECG and echocardiography may show atrial fibrillation/flutter, low QRS voltage, dilated cardiomyopathy, and tricuspid and mitral regurgitation. The gastrointestinal form is usually diagnosed by barium esophagogram (esophageal dilatation) and barium enema (megacolon of the sigmoid and rectum).

Treatment

Two drugs are recommended for the treatment of acute phase Chagas disease: nifurtimox and benznidazole. These drugs are usually associated with severe side effects: (a) nifurtimox may cause weight loss, anorexia, behavioral changes, and an antabuse effect; (b) benznidazole may cause rashes, bone marrow suppression, and peripheral neuropathy. Other classes of drugs, such as allopurinol and itraconazole, have been shown to halt the progression into cardiomyopathy, although no form of treatment has been shown to reverse damage caused by the disease. Newer drugs such as triazole derivatives (posaconazole, ravuconazole) and cruzipain inhibitors (a parasite protease) are currently under development.

Symptom-specific management is used to treat patients with chronic manifestations. Cardiac manifestations are controlled by pacemakers and antiarrhythmic drugs, such as amiodarone, while megasyndromes are managed with special diets, laxatives, and surgical procedures.

Epidemiology

Chagas disease is estimated to have infected more than 10 million people worldwide. Most cases are reported in the Latin Americas, where more than 25 million people are at risk for the disease. Serologic techniques have identified that up to 13% of populations in certain endemic regions are positive for *T. cruzi* antibodies. An estimated 10,000 to 12,000 people die of the disease annually.

The prevalence and distribution of American trypanosomiasis has been continually shifting. Endemic regions include most of Central America and the southern cone of South America. Several factors tend to determine the changes in prevalence of the disease in endemic countries. In certain regions in Mexico, delayed control policies and mobilization probably contributed to an increase in prevalence. In the past 25 years, there have been several international efforts to control and prevent the disease in these endemic areas, most notably vector control strategies, and improved blood transfusion safety regulations. Brazil, Chile, Uruguay, and several areas of Argentina and Paraguay have eliminated the vector *Triatoma infestans*. However, the disease persists due to emergence of secondary domestic vectors, and vector resistance to insecticides.

Initially thought to be confined within the Latin American region, countries such as USA, Canada, Spain, France, Switzerland, Japan, and Australia have seen a number of cases, primarily due to human migration patterns as well as from blood transfusion, organ donation and vertical transmission. However, these countries are still regarded as non-endemic, and majority of the cases are attributed to imported infections from endemic areas.

Chagas disease is included in the WHO list of Neglected Tropical Diseases (NTDs), and is the leading cause of parasite-related deaths in Latin America. In 2003, it ranked 3rd as the leading cause of parasitic infection in the world, behind malaria and schistosomiasis.

Prevention and Control

There have been major breakthroughs in the control and prevention of American trypanosomiasis, particularly by Brazil, Chile, and Uruguay. Vector control and blood transfusion regulations have delivered positive outcomes, in terms of disease prevention in these countries. Spraying of insecticides, use of insecticide-treated bed nets, and house improvements to prevent vector infestation

have been proven cost-effective. International organizations such as the WHO and the manufacturers of the antiparasitic drugs are working in tandem to ensure the availability of drugs for the treatment of the disease.

In Mexico and non-endemic countries near endemic countries, the coverage, quality and safety of blood transfusion screening is being evaluated as avenues for disease prevention. Vaccine development has not yet been successful, but the advent of newer technologies and characterization of the *T. cruzi* genome may aid in future vaccine research.

Trypanosoma brucei gambiense
Trypanosoma brucei rhodesiense

Human African trypanosomiasis (HAT), also known as African sleeping sickness, is a

highly fatal disease caused by two subspecies of *Trypanosoma brucei*: *T. brucei gambiense* and *T. brucei rhodesiense*. A third subspecies, *T. brucei brucei*, primarily affects wild and domestic animals; collectively, the three subspecies represent the *Trypanosoma brucei* complex. The earliest reports of sleeping sickness in Africa date back to 1734, while the formal correlations between the symptoms, the parasite in blood and CSF, and the relationship between the parasite and its insect vector were established during the early 1900s.

Parasite Biology

Members of the *T. brucei* complex belong to the trypanosome family Salivaria. The parasite is usually transmitted via the bite of the blood-sucking tsetse fly (*Glossina* spp.) feeding from an infected mammalian host (Figure 2.25). Since

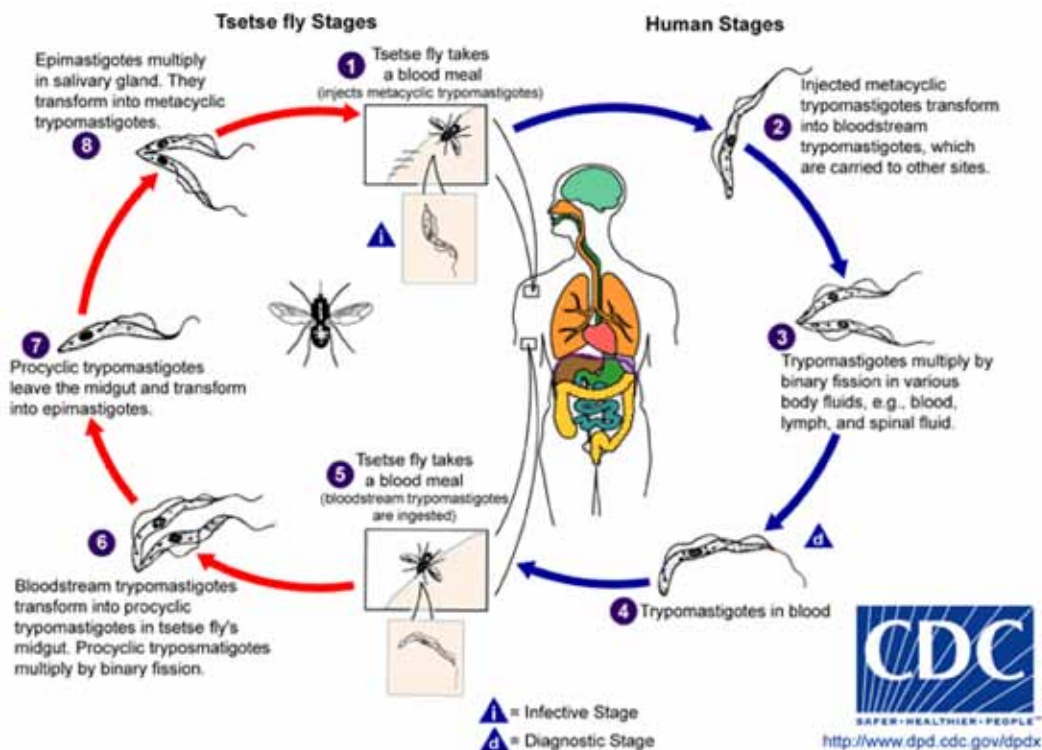


Figure 2.25. Life cycle of *Trypanosoma brucei*
 (Accessed from www.dpd.cdc.gov/dpdx)

the disease relies heavily on the tsetse fly for its transmission, HAT cases are localized in regions of sub-Saharan Africa, primarily in remote rural areas where tsetse fly habitats are located.

T. brucei gambiense is localized mostly in the western and central regions of sub-Saharan Africa. It primarily affects humans, but utilizes dogs, pigs, and sheep as reservoir hosts. It is responsible for the chronic type of sleeping sickness, and accounts for 95% of all HAT cases.

T. brucei rhodesiense is found in east Africa and is primarily a zoonosis of cattle and wild animals, with humans being accidental hosts. It causes the more acute and rapidly fatal form of sleeping sickness, and accounts for the remaining 5% of HAT cases.

Only the epimastigote and trypomastigote forms are exhibited by the *T. brucei* complex. The trypomastigotes are polymorphic: there are typical slender forms, and short, stumpy forms. They are flattened and fusiform in shape, 14 to 33 μm in length and 1.5 to 3.5 μm in width. The body tapers anteriorly and is blunt posteriorly. The centrally located nucleus contains a large central karyosome. There is an undulating membrane, and a single flagellum that runs along the edge of the undulating membrane and becomes free anteriorly.

Once ingested by the intermediate host, *Trypanosoma brucei* trypomastigotes undergo several developmental changes from trypomastigote into procyclic forms in the insect's midgut. After multiplying for 15 to 20 days, the epimastigotes migrate to the foregut into the insect's salivary glands, where they mature into metacyclic trypomastigotes. When the infected fly bites another mammalian host, these infective trypomastigotes are injected into the new host where they multiply and mature in blood and connective tissue. In humans, *T. brucei* lives in the blood, in the reticular tissue of lymph and spleen, and the CSF. The long, slender trypomastigotes multiply by longitudinal binary fission.

Though mostly transmitted through its insect vector, the disease can also be transmitted

through mechanical methods (accidental needle pricks, other blood sucking insects), as well as vertically, via mother-to-child infection through the placenta.

Pathogenesis and Clinical Manifestations

Human African trypanosomiasis has two types, acute and chronic, depending on the subspecies causing the disease. *Trypanosoma brucei gambiense* sleeping sickness manifests months or years after initial infection, while symptoms of *T. brucei rhodesiense* sleeping sickness may appear just weeks after infection.

The initial lesion of African trypanosomiasis begins as a local, painful, pruritic, erythematous chancre located at the bite site, progressing into a central eschar, and resolving after 2 to 3 weeks. This trypanosomal chancre is more common in Gambian sleeping sickness. Several days after the development of the chancre, usually within 3 to 10 days, the next stages of the disease manifest.

Both types of HAT undergo two stages: early and late. During the early phase of HAT, called the hemolymphatic stage, the parasites proliferate in the bloodstream and lymphatics. The patient may manifest with irregular bouts of fever, headache, joint and muscle pain, and malaise. Anemia, myocardial inflammation, disseminated intravascular coagulation, and renal insufficiency may occur. Frequently, in Gambian trypanosomiasis, the posterior cervical lymph nodes are enlarged, non-tender, and rubbery in consistency (Winterbottom's sign). Other lymph nodes, such as axillary and supraclavicular lymph nodes, may also be involved in both types of sleeping sickness. The signs and symptoms manifested within this phase are due to tissue damage, either from parasitic toxins or immune complex reactions that target organs and RBCs. The early systemic phase lasts from 1 to 6 months.

The late phase of the disease, known as the meningoencephalitic stage, marks the involvement of the central nervous system. The brain and meninges become involved

as the parasites find their way into the CNS through the bloodstream. This usually occurs 3 to 10 months after initial infection in Gambian infections, but can manifest after just a few weeks in Rhodesian trypanosomiasis. Neurologic symptoms become evident, such as apathy, behavioral changes, headache, and sleep pattern changes. These may be followed by more severe symptoms, such as convulsions, tremors, speech defects, disturbances in speech and reflexes, and even paralysis. Kerandel's sign may manifest as a deep, delayed hyperesthesia (delayed bilateral pain out of proportion to the extent of tissue injury). In the later stages, somnolence manifests, followed by a deep coma. Death eventually follows either from the disease itself, or from intercurrent infection due to immunosuppression.

Areas of the CNS usually involved in the meningoencephalitic phase include the frontal lobes, pons, medulla, and perivascular areas. Parasites may also be seen in the CSF. Autopsy of HAT patients reveals edema and numerous, small, and confluent hemorrhages.

Trypanosomes are able to evade the immune response of the host through a process called antigenic variation. This refers to the ability of the trypomastigote to continuously change its surface coat, composed of variant surface glycoproteins, so that the host's antibodies cannot recognize the parasite in subsequent recurrent waves of parasitemia.

Diagnosis

Diagnosis of human African trypanosomiasis depends upon the demonstration of highly motile trypomastigotes in expressed fluid from a chancre, lymph node aspirate, and CSF. Thick and thin blood films can be stained with Giemsa. Buffy coat concentration method is recommended to detect parasites when they occur in low numbers. Examination for trypomastigotes is usually done during the hemolymphatic stage of the disease, and is more useful for the diagnosis of *T. brucei rhodesiense*

due the relative higher levels of parasitemia. Serial examinations may be necessary due to varying levels of parasitemia. Other diagnostic techniques include enzyme-linked immunosorbent assay, immunofluorescence, indirect hemagglutination test, mini-anion exchange centrifugation technique, and PCR.

CSF examination is mandatory in patients with suspected HAT to detect CNS involvement. Abnormal CSF findings include increase in cell count, opening pressure, protein concentration, and IgM levels. The latter is considered pathognomonic for the meningoencephalitic stage of the disease.

Card agglutination test for trypanosomiasis (CATT) detecting circulating antigens in persons infected with *T. brucei* complex is available commercially and can be used in the field setting to screen at-risk populations. This technique provides a rapid and highly specific method of screening for HAT cases; however, the method has low sensitivity for certain strains of *T. brucei gambiense* in certain areas of West Africa.

Treatment

Treatment of African sleeping sickness depends on the stage of the disease. For the first stage, intravenous suramin sodium for both *T. brucei gambiense* and *T. brucei rhodesiense*, and intramuscular pentamidine for the Gambian form can be used. These drugs have side effects, which include fever, rash, renal insufficiency, muscle pain, and paresthesia for suramin; and tachycardia, hypotension, and hypoglycemia for pentamidine. These drugs do not cross the blood-brain barrier, and so, they cannot be used for the CNS stage of the disease.

Once CNS involvement occurs, intravenous melarsoprol is the drug of choice for both types of sleeping sickness. This arsenic-containing drug can cause fatal arsenic encephalopathy (usually prevented by co-administration of corticosteroids), and resistance to the drug has also been observed. A febrile episode

called a Jarisch-Herxheimer reaction due to trypanosome lysis may occur following melarsoprol treatment.

A second-line drug, nitrofurazone, is used in cases of melarsoprol treatment failure. A newer drug, eflornithine, is less toxic than melarsoprol, and can also be used during the hemolymphatic stage; however, it is only effective against *T. brucei gambiense*. Recent evidence has shown that a combination treatment of oral nifurtimox and intravenous eflornithine is of similar efficacy compared to longer intravenous monotherapy with either agent. Combination therapy is advantageous due to the relative ease in administration, and a decreased risk of developing drug resistance. Although nifurtimox is currently registered as a drug against American trypanosomiasis, its use in the nifurtimox-eflornithine combination treatment (NECT) has been included in the WHO List of Essential Medicine.

Epidemiology

Sleeping sickness affects around 300,000 to 500,000 people in 36 countries within sub-Saharan Africa. It is estimated that more than 50 million people are at risk of infection. In the last 10 years, most reported cases came from the Democratic Republic of Congo (DRC), followed by the Central African Republic. Other countries such as Angola, Cameroon, Chad, Congo, Côte d'Ivoire, Equatorial Guinea, Gabon, Guinea, Kenya, Malawi, Nigeria, Sudan, Uganda, United Republic of Tanzania, Zambia, and Zimbabwe have also reported cases.

During the turn of the century, between 10,000 and 40,000 annual cases of HAT were being reported. However, newer data from the WHO has estimated more recently that new cases have dropped below the 10,000 mark, a first in 50 years.

Tsetse flies live near the banks of rivers and streams, therefore transmission can readily occur when people frequent these areas to swim and

do their laundry. Rhodesian trypanosomiasis is an occupational hazard for persons working in game reserves, and may also be a threat to visitors of game parks. Cattle and game animals like antelopes can serve as reservoir hosts for the parasite.

Prevention and Control

Vector control is the primary method used in the control and prevention of African sleeping sickness. Tsetse fly trapping is the main strategy employed to decrease the vector population. Use of insecticides and protective clothing are recommended to prevent contact with the insect vector. Regulation and treatment of reservoir hosts such as cattle and game animals are also being looked upon as an effective means of preventing disease transmission.

Several programs developed to eliminate the insect vector have been in place in Africa. The Kwando-Zambesi Regional Tsetse Eradication project started in Botswana, and in 2000, the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was established. Aerial and localized spraying of insecticides in Angola, Botswana, Namibia, and Zambia has eradicated the tsetse fly in these African countries.

The WHO has established partnerships with private companies such as Aventis Pharma (Sanofi-Aventis) and Bayer HealthCare to provide surveillance and management support to endemic countries.

Leishmania spp.

Early descriptions of leishmaniasis have been found as early as the first century A.D., where American Indians documented the disease in pottery figures. Cunningham studied the “Delhi boil” in India back in 1885, and Leishman had properly identified the intracellular parasites in 1903. *Leishmania braziliensis* was later identified in 1911 by Gaspar Viana, as was the insect vector which transmitted the parasite in 1922 by Henrique Aragao.

Parasite Biology

Leishmaniasis is a disease caused by infection of the diploid protozoa belonging to the genus *Leishmania*. This genus is actually divided into two subgenera, differentiated from one another by the location of their development inside the insect vector, as well as the areas in which they are endemic. Currently there are about 15 species of *Leishmania* which cause clinical manifestations in humans. This diverse pool of different species is historically divided and classified based on their biological, clinical, geographic, and epidemiological characteristics.

Epidemiologically, the *Leishmania* spp. are divided into Old World and New World leishmaniasis. In the Old World, the common species involved are *L. tropica* (Asia and Eastern Europe), *L. aethiopica* (Africa) and *L. major*. New World leishmaniasis affects

Mexico, Central America, and some parts of South America, as well as the Amazon rain forest, and is usually caused by *L. mexicana*, *L. amazonensis*, *L. guyanensis*, *L. braziliensis*, and *L. chagasi*. Arthropods, particularly sandflies of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World), act as the insect vector for these parasites. Dogs are the primary reservoir in urban areas, and rodents also act as reservoirs in both urban and rural areas.

Leishmania spp. produce amastigotes intracellularly in the mammalian host, and promastigotes in the hindgut (*Viannia* subgenus), midgut (*Viannia* and *Leishmania* subgenera), and proboscis (*Viannia* and *Leishmania* subgenera) of the insect vectors. Amastigotes are ovoid or rounded bodies measuring 2 to 3 μm in length and live intracellularly in monocytes, polymorphonuclear leukocytes, or endothelial cells. The nucleus is large, while an axoneme

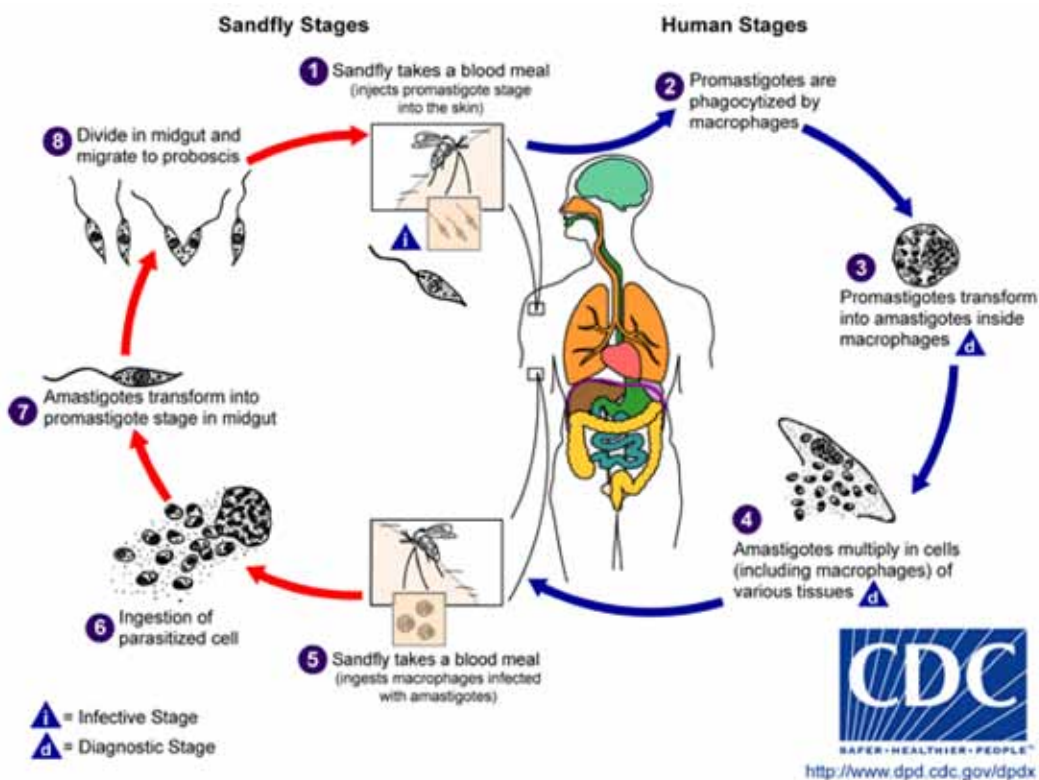


Figure 2.26. Life cycle of *Leishmania* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

arises from the kinetoplast and extends to the anterior tip.

Promastigotes have a single free flagellum arising from the kinetoplast at the anterior end. They measure 15 to 20 μm in length and 1.5 to 3.5 μm in width. The infective promastigotes in the proboscis of the sandfly are injected into the host's skin during feeding (Figure 2.26). They then invade the cells of the reticuloendothelial system, transform into amastigotes, and multiply via binary fission. When the parasitized cell ruptures, the amastigotes that are released either invade new cells, or are taken up by sandflies during feeding, where they transform into promastigotes in the gut, multiply by binary fission, and migrate to the foregut.

Leishmania spp. may also be transmitted congenitally, through blood transfusion, by contamination of bite wounds, and by direct contact with contaminated specimens.

Pathogenesis and Clinical Manifestations

Clinically, leishmaniasis can be divided into four categories: cutaneous leishmaniasis (CL), diffuse cutaneous leishmaniasis (DCL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). The wide spectrum of symptoms manifested by leishmaniasis is often compared to leprosy, where the localized CL is similar to tuberculoid leprosy, and DCL is similar to lepromatous leprosy.

The immune response of the host against the infection depends on *Leishmania*-specific Th1-type CD4⁺ T-cells, macrophages, and cytokines. However, other factors such as genetics, nutritional status, and environmental factors may affect the outcome of infection.

A. Cutaneous Leishmaniasis

Cutaneous leishmaniasis is the most common form of the disease, and is caused by several species of *Leishmania*, including *L. tropica* (dry or urban oriental sore), *L. major* (moist or rural oriental sore), and *L. mexicana* (chiclero ulcer, usually affecting the ears). The

incubation period ranges from two weeks to several months. An erythematous papule or nodule, called an "oriental button," is produced at the inoculation site. The lesion has raised edges and a central crater. During the course of several weeks, the papule forms a violaceous ulcer as it enlarges in size. The lesion may heal spontaneously after a few months, leading to a disfiguring scar; in the case of New World leishmaniasis, CL may progress to other forms of leishmaniasis.

B. Diffuse Cutaneous Leishmaniasis

The manifestation of DCL, also called anergic or lepromatous leishmaniasis, is characterized by a localized, non-ulcerating papule, eventually developing numerous diffuse satellite lesions that affect the face and extremities. This type of leishmaniasis may be initially diagnosed as lepromatous leprosy.

C. Mucocutaneous Leishmaniasis

Mucocutaneous leishmaniasis develops in about 2 to 5% of persons infected with *L. braziliensis*, either concurrently or even several years after the resolution of skin lesions. It may be also due to the contiguous spread of cutaneous leishmaniasis caused by *L. tropica*. Involvement of the mucous membranes of the nasal and oral cavities results in nasal stuffiness, discharge, epistaxis, and destruction of the nasal septum. This disfiguration is often called *espundia*. Progression into the pharynx and larynx may threaten the airway passage, and may lead to dysphonia, dysphagia, and even aspiration pneumonia.

Lesions usually manifest with few parasites. Systemic Th1 response is strong in cases of MCL, with increased levels of peripheral mononuclear cells in the blood.

D. Visceral Leishmaniasis

Visceral leishmaniasis (or *kala azar*), is a disseminated parasitosis primarily caused by *L. donovani* complex: *L. donovani*, *L. chagasi*, and

L. infantum. It has an incubation period of 2 to 8 months, but clinical symptoms in previously infected but asymptomatic persons may appear during immunocompromised states. This manifestation of the disease stems from the spread of parasites into the bone marrow, spleen, and liver.

In the acute phase, twice-daily fever spikes (double quotidian), with accompanying chills may be present, which might be mistaken for malaria. During the subacute and chronic course, common signs and symptoms include fever, weakness, loss of appetite, weight loss, hemorrhage, and abdominal enlargement associated with hepatosplenomegaly.

Phagocytosed amastigotes are present only in small numbers in the blood. However, they are numerous in the reticuloendothelial cells of the spleen, liver, lymph nodes, bone marrow, intestinal mucosa, and other organs. In patients with VL, *Leishmania*-specific Th1 response is usually low or absent. VL, if left untreated, has a greater than 95% mortality rate.

Post-*kala azar* dermal leishmaniasis (PKDL) is a sequela of visceral leishmaniasis, usually seen in endemic areas. It manifests as a cutaneous eruption resulting in hypopigmented macules, malar erythema, nodules, and ulcerations. These lesions usually manifest a few months to several years after treatment.

Diagnosis

Diagnosis of active leishmaniasis is based on the microscopic demonstration of *Leishmania* from lesion and tissue scrapings, aspirates, or biopsy. Giemsa and hematoxylin-eosin stains are often used in microscopic and histologic samples, and the demonstration of amastigotes confirms the diagnosis of leishmaniasis. Cultures are unreliable due to the difficulty of isolating the parasites, especially in old lesions. There are however reports of successful primary isolation of the New World cutaneous leishmania using the Novy, MacNeal, and Nicolle medium (NNN). The Schneider's medium was also

found useful. Animal inoculation using hamsters could detect low intensity of infection.

The leishmanin skin test (Montenegro skin test) can be used to identify exposure to the parasite. It is usually positive in cases of CL and MCL, but is negative in cases of DCL and *kala azar*.

Immunologic assays such as ELISA and rk39 antigen dipstick test have demonstrated high sensitivity and specificity for VL in certain immunocompetent patient populations. Direct agglutination, urine antigen assays, and newer techniques such as flow cytometry and molecular diagnostic modalities (polymerase chain reaction, RFLP analysis) are also being used; the latter may be used to identify the species of *Leishmania*.

Treatment

Primary pharmacologic treatment is based on antimony compounds, notably the pentavalent antimonials: sodium stibogluconate and n-methyl-glucamine (meglumine). These drugs are still being used in areas where susceptibility is still good, due to its low cost. However, primary treatment failure and relapses are often observed using these drugs, especially in patients with AIDS. Side effects such as abdominal pain, nausea, arthralgia, and even fatal arrhythmias are high using these drugs, and treatment should only be done after consultation with infectious disease experts. Treatment with the antimonial drugs requires daily intramuscular or intravenous administration for up to 4 weeks, and hospital confinements are necessary.

In cases where there is treatment failure with antimonials, or in areas where resistance is high, intravenous amphotericin B is the drug of choice. Amphotericin B has a high cure rate; however, the associated side effects, as well as the cost and availability of the drug are significant limiting factors. Lipid-based preparations of the drug (AmBisome) are currently being utilized as a highly effective, better tolerated, and overall

cost-effective drug formulation for cutaneous and visceral leishmaniasis.

In India, where sodium pentavalent antimony resistance is high, the antineoplastic drug miltefosine was introduced in 2002 to treat VL. Miltefosine is the only oral drug currently given to VL patients.

Pentamidine is another second-line drug for cutaneous as well as the visceral form of the disease. However, due to side-effects and the development of drug resistance, pentamidine use has been limited. For the cutaneous form of leishmaniasis, topical paromomycin has shown efficacy in certain areas.

Combination therapy using two or more of the anti-leishmanial drugs is being studied. The presence of drug resistance especially towards the pentavalent antimonials, poor treatment outcomes of complicated cases (such as HIV coinfection), the potential for greater efficacy, better compliance, and fewer side effects are reasons why combination therapy for VL is the current consensus. Among the drug combinations currently being used or under clinical trials are: sodium stibogluconate plus paromomycin, and liposomal amphotericin B plus either miltefosine, or sodium stibogluconate.

Epidemiology

Leishmaniasis is a global disease distributed across 88 countries in four continents. It affects more than 12 million people worldwide, and more than 350 million are at risk for the disease. New cases of cutaneous leishmaniasis number between 1 to 1.5 million per year, the majority of which occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia, and Syria. American soldiers deployed in Afghanistan and Iraq have also demonstrated cases of CL. Mucocutaneous leishmaniasis occurs in Bolivia, Brazil, and Peru, while half a million new cases annually of visceral leishmaniasis occur primarily in Bangladesh, Brazil, India, Nepal, and Sudan. In 2009, there was a noted upsurge in VL cases in Sudan compared to previous years, affecting

mostly poor and malnourished children below 15 years old.

Leishmaniasis is primarily a disease of poverty. It affects people living in squalid conditions, and is associated with poor housing, malnutrition, a weak immune system, and lack of resources. Environmental changes such as deforestation, new irrigation schemes, and urbanization are also linked to changes in the epidemiology of the disease. In urban areas where leishmaniasis occurs, there is a greater epidemic threat.

Visceral leishmaniasis is an important opportunistic infection in AIDS patients. VL/HIV co-infection is currently a major threat in the control and prevention of either disease. Immunosuppression from HIV predisposes to VL, while VL infection accelerates HIV replication and progression to AIDS. VL/HIV co-infection has been documented in 35 countries, with most cases coming in from Ethiopia, southern Europe (Spain, Italy, France, and Portugal), and Brazil.

In the Philippines, there have been imported cases of cutaneous lesions referred to the University of the Philippines—College of Public Health, where amastigotes were identified from the patients.

Prevention and Control

Preventive measures against leishmaniasis include usage of insect repellants containing DEET and permethrin, insecticide-treated clothing, and fine-mesh bed nets. Use of fine mesh screens and spraying of houses and buildings are also being done in certain areas. However, interval spraying predisposes to resistance of sandflies to the insecticides, not to mention the impact of insecticides on the environment.

Regulation of reservoir hosts is another important aspect in the control and prevention of leishmaniasis. Insecticide-treated dog collars, mass testing of domestic dogs, and even extermination of infected dogs are current

strategies that address zoonotic transmission of the disease.

At present, there is no commercially available form of either active or passive chemoprophylaxis against leishmaniasis. However, in immunocompetent individuals, a form of immunity persists after resolution of active lesions. Certain countries, such as endemic areas in the Middle East, have been using live parasites either from infected insect vectors, or in recent years, from cultures, to inoculate inconspicuous areas (such as the buttocks) so as to protect themselves from disfiguring facial lesions from future infections. Commercial vaccines are currently under development.

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CHAPTER 3

Nematode Infections

Intestinal Nematodes

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes

Ascaris lumbricoides

The most common intestinal nematode of man is *Ascaris lumbricoides* or the giant round worm, which occurs most frequently in the tropics. It is estimated that more than 1 billion individuals are infected, 70% of whom are from Asia.

Ascaris is a soil-transmitted helminth (STH), along with *Trichuris trichiura* and hookworms, which means that the soil plays a major role in the development and transmission of the parasite. It causes varying degrees of pathology: (a) tissue reaction to the invading larvae, (b) intestinal irritation to the adult, and (c) other complications due to heavy infection and extraintestinal migration. STH infections are diseases of poverty, and contribute to malnutrition and impairment of cognitive performances. They, likewise, reduce work capacity and productivity of adults.

Parasite Biology

This worm has a so-called “polymyarian type” of somatic muscle arrangement in which cells are numerous and project well into the body cavity. The whitish or pinkish worms are large, with males measuring 10 to 31 cm and females 22 to 35 cm in length, with smooth striated cuticles. The worms have a terminal mouth with three lips and sensory papillae. Males have a ventrally curved posterior end with

two spicules. Females have paired reproductive organs in the posterior two-thirds, while males have a single, long, tortuous tubule. The adults reside in but do not attach to the mucosa of the small intestines. Larval morphology is similar to the adult. *Ascaris* has been shown to produce pepsin inhibitor 3 (PI-3) that protects worms from digestion and phosphorylcholine that suppresses lymphocyte proliferation.

The infertile eggs (Plate 3.1a) measure 88 to 94 μm by 39 to 44 μm , longer and narrower than fertile eggs, with a thin shell and irregular mammilated coating filled with refractile granules. These infertile eggs may be difficult to identify and are found not only in the absence of males. They are found in about two of five infections.

Fertile eggs measure 45 to 70 μm by 35 to 50 μm (Plate 3.1b). There is an outer, coarsely mammilated albuminous covering which may be absent or lost in “decorticated” eggs. The egg has a thick, transparent, hyaline shell with a thick outer layer as a supporting structure and a delicate vitelline, lipoidal, inner membrane, which is highly impermeable. At oviposition, the fertile eggs have an ovoid mass of protoplasm, which will develop into larvae in about 14 days.

The infective stage is the fully embryonated egg (Plate 3.1c). When these eggs are ingested, they hatch in the lumen of the small intestine, releasing the larvae. The larvae then migrate

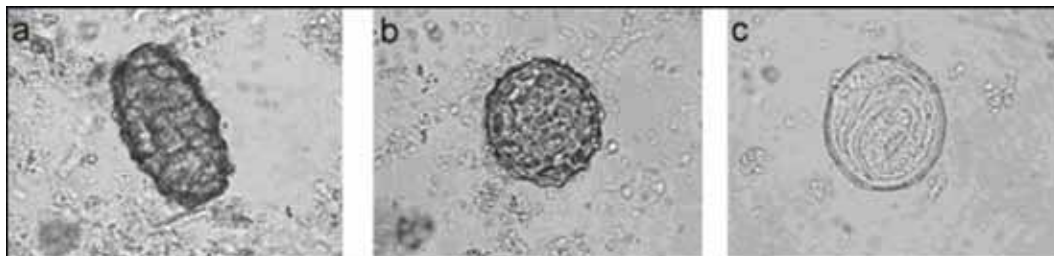


Plate 3.1. *Ascaris* unfertilized egg (a), fertilized egg (b), and embryonated egg (c)
(Courtesy of the Department of Parasitology, UP-CPH)

to the cecum or proximal colon where they penetrate the intestinal wall. These larvae enter the venules to go to the liver through the portal vein, on to the heart and pulmonary vessels where they break out of capillaries to enter the air sacs. In the lungs, larvae undergo molting before migrating to the larynx and oropharynx to be swallowed into the digestive tract. This hepato-tracheal migration phase takes about 14 days, while the development of egg-laying adult worms takes about 9 to 11 weeks after egg ingestion. The life span of an adult worm is about 1 year.

A female *Ascaris* produces about 200,000 eggs per day, but this number decreases with increasing worm load. The eggs are deposited in the soil when a person with *Ascaris* infection defecates indiscriminately. In the soil, it takes about 2 to 3 weeks for eggs to develop into the infective stage (embryonation) under favorable conditions with suitable temperature, moisture, and humidity. The larvae undergo two molts to reach their 3rd stage within the egg and become embryonated. Only when this infective egg is swallowed can humans become infected with *Ascaris* (Figure 3.1). The embryonated eggs can survive in moist shaded soil for a few months to about two years in tropical and sub-tropical areas, but for much longer in temperate regions.

Pathogenesis and Clinical Manifestations

A majority of *Ascaris* infections are asymptomatic, although an estimated 120 to 220 million cases exhibit morbidity as a result

of the infection. Ascariasis was estimated to have contributed to a total of 1.85 million disability-adjusted life years (DALYs) in 2004.

The varied pathology of ascariasis includes the reaction of tissues to invading larvae, irritation of the intestine by the mechanical and toxic action of the adult, and complications arising from the parasite's extraintestinal migration (Plates 3.2–3.4). The usual infection of 10 to 20 worms may not show symptoms, hence, may go unnoticed by the host unless it is discovered by stool examination or the spontaneous passing of worms in the stool.

During lung migration, the larvae may cause host sensitization resulting in allergic manifestations such as lung infiltration, asthmatic attacks, and edema of the lips. Symptoms of difficulty of breathing and fever similar to pneumonia may occur as a result of penetration by several larvae through the lung capillaries as they enter the air sacs. The most frequent complaint of patients is vague abdominal pain. Eosinophilia is present during larval migration. Moderate infections may produce lactose intolerance and vitamin A malabsorption. Heavy infections are likely to cause bowel obstruction (due to bolus formation), intussusception, or volvulus that may result in bowel infarction and intestinal perforation.

Serious, and at times, fatal effects of ascariasis are due to erratic migration of adult worms. They may be regurgitated and vomited, may escape through the nostrils or rarely, inhaled

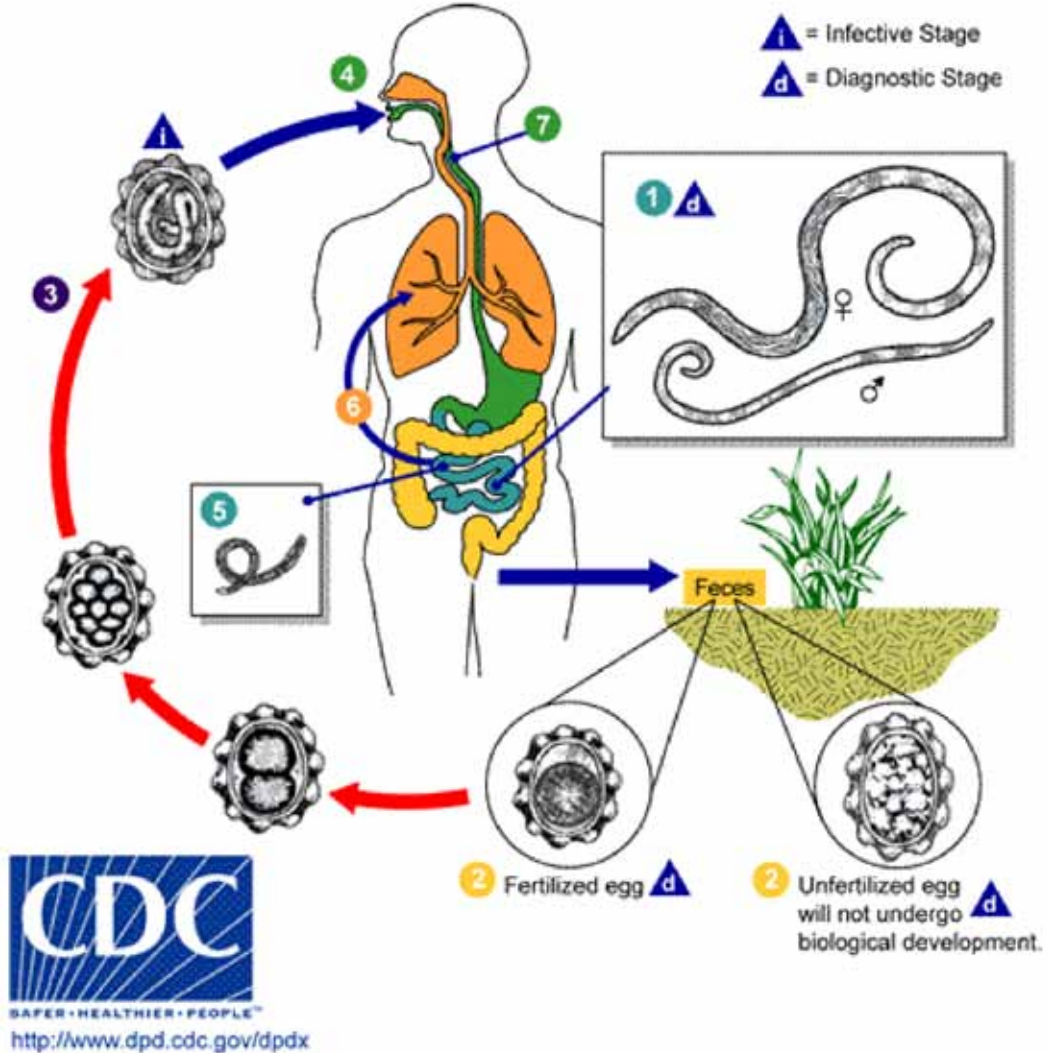


Figure 3.1. Life cycle of *Ascaris lumbricoides*
(Accessed from www.dpd.cdc.gov/dpdx)

into the trachea. The worms may invade bile ducts through the ampulla of Vater and enter the gallbladder or liver. Patients with biliary ascariasis experience severe colicky abdominal pain, which is brought about by the movement of the worms inside the biliary tract. Worms may also lodge in the appendix or occlude the pancreatic duct and cause acute appendicitis or pancreatitis, respectively. Intestinal bacteria may be carried to these migration sites producing

abscesses. Penetration of the worms through the intestinal wall into the peritoneal cavity may occur and result in either acute peritonitis or chronic granulomatous peritonitis.

Complications brought about by the larvae and adult worms are a cause for concern. The continuous biting or pricking of the intestinal mucosa for food by a few *Ascaris* adults may irritate nerve endings in the mucosa and result in intestinal spasm leading to intestinal

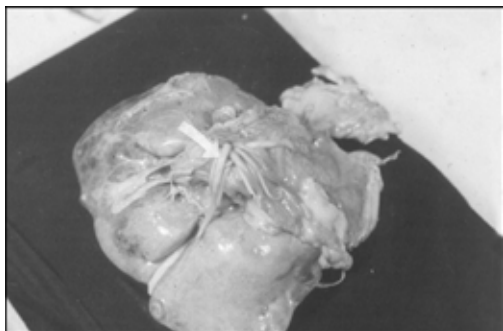


Plate 3.2. *Ascaris* in the liver
(Courtesy of Dr. Benjamin Cabrera)

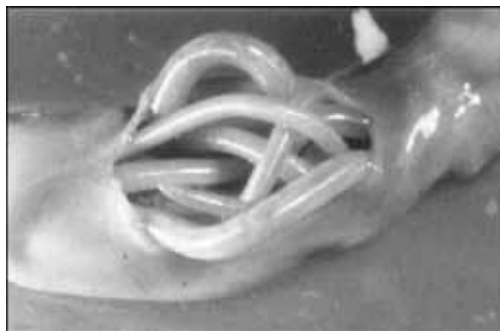


Plate 3.3. Intestinal obstruction with *Ascaris*
(Courtesy of Dr. Benjamin Cabrera)



Plate 3.4. *Ascaris* in the brain
(Courtesy of Dr. Benjamin Cabrera)

obstruction. Hence, a child need not harbor hundreds of *Ascaris* adults to produce intestinal obstruction.

Diagnosis

Clinical diagnosis of ascariasis is rather inaccurate because the signs and symptoms are quite vague and are indistinguishable from those of other intestinal nematode infections or from non-parasitic infections. Hence, the clinical diagnosis of ascariasis should be confirmed or established by microscopic examination of a stool sample. The disease should be highly suspected in a child who reportedly passed out the worm with his feces.

In the laboratory, direct fecal smear (DFS), Kato thick Smear, Kato-Katz techniques, as well as concentration techniques, such as formalin-ether/ethyl acetate concentration technique (FECT), are stool examination techniques used to diagnose ascariasis by confirming the presence of eggs in the feces.

DFS is less sensitive compared to the Kato thick Smear and Kato-Katz techniques. The last two methods are useful for both individual and mass screening in schools or in the community. Kato-Katz technique also provides quantitative diagnosis in terms of the intensity of helminth infection in eggs per gram (epg) of stool that is useful in monitoring the efficacy of treatment in clinical trials, as well as public health programs. A study in China comparing the sensitivity of different diagnostic techniques for helminth infections showed that Kato-Katz had a sensitivity of 98%, while sodium acetate-acetic acid-formalin (SAF) concentration technique had a sensitivity of 93% for the diagnosis of *Ascaris* infections. In a local study, the sensitivity for the detection of *Ascaris* through single and double Kato-Katz stool sample/s were 96.9% and 99.9%, respectively. In addition, in a local study comparing the sensitivity of DFS and FECT for the screening of food handlers, FECT was shown to have a higher sensitivity and detection rate for intestinal parasite infections compared with DFS.

Treatment

Individual infections are cured by a single dose of any of the broad-spectrum anthelmintics such as albendazole, mebendazole, and pyrantel pamoate. A recent systematic review and meta-analysis revealed that a single-dose oral albendazole, mebendazole, and pyrantel pamoate had cure rates of 93.9%, 96.5%, and 87.9%, respectively. Albendazole is given at 400 mg single dose (200 mg for children 12-23 months), mebendazole at 500 mg single dose, and pyrantel pamoate at 10 mg/kg (max. 1 g) also as a single oral dose. Ivermectin has been shown to be as effective as albendazole if given at a dose of 200 µg/kg single dose. Nitazoxanide may be given at 500 mg twice a day for 3 days (100 mg twice a day for 3 days for children 1-3 years old; 200 mg twice a day for 3 days for children 4-11 years old).

Benzimidazoles, such as albendazole and mebendazole, bind to the parasites' b-tubulin resulting in the disruption of parasite microtubule polymerization. This binding eventually results in the death of adult worms that takes several days. Adverse reactions to these anthelmintics are rare, mild, and transient. These are epigastric pain, headache, diarrhea, nausea, vomiting, and dizziness, among others. These reactions may be minimized by administering the deworming tablet after a meal.

In 2001, the World Health Assembly recommended preventive chemotherapy among high risk groups (e.g., preschool- and school-age children) for morbidity control in communities where the cumulative prevalence of STH infections is greater than 20%. Preventive chemotherapy is done through mass drug administration (MDA) with anthelmintics, either alone or in combination, among target populations, even without the benefit of stool examination. The World Health Organization (WHO) recommends coverage of at least 75% of the target populations during MDA. In the Philippines, MDA, as part of the

Integrated Helminth Control Program (IHCP) of the Department of Health (DOH), is being conducted in elementary schools every January and July for school-age children through the Department of Education (DepEd). MDA for preschool-age children is being conducted under the *Garantisadong Pamabata* program through the DOH and the local government units. In filariasis endemic areas, MDA with albendazole and diethylcarbamazine every November also contribute to the control of STH. The IHCP targets an MDA coverage of at least 85% of the target population.

The WHO recommends targeting other high-risk groups such as women of child-bearing age and pregnant women. Pregnant women in their 2nd or 3rd trimester, as well as lactating women may receive albendazole or mebendazole. Children less than one year old and pregnant women in their first trimester are ineligible for MDA with albendazole or mebendazole.

Recent studies have revealed that the benefits of regular deworming in the school-age group include improvements in iron stores, growth and physical fitness, cognitive performance and school attendance. In younger children, studies have shown improved nutritional indicators such as reduced wasting, stunting, and improved appetite.

Use of anthelmintics to control helminth infections in livestock resulted in anthelmintic resistance to all drug classes. Although there have been a few reports on the reduced efficacy of anthelmintics in humans, these reports were unable to show evidence of genetically transmitted drug resistance. Currently, drug resistance monitoring involves the identification of molecular or genetic markers for resistance specific to each of the anthelmintic drug classes.

Epidemiology

Ascaris has a cosmopolitan distribution (Figure 3.2). About 1.2 billion people globally

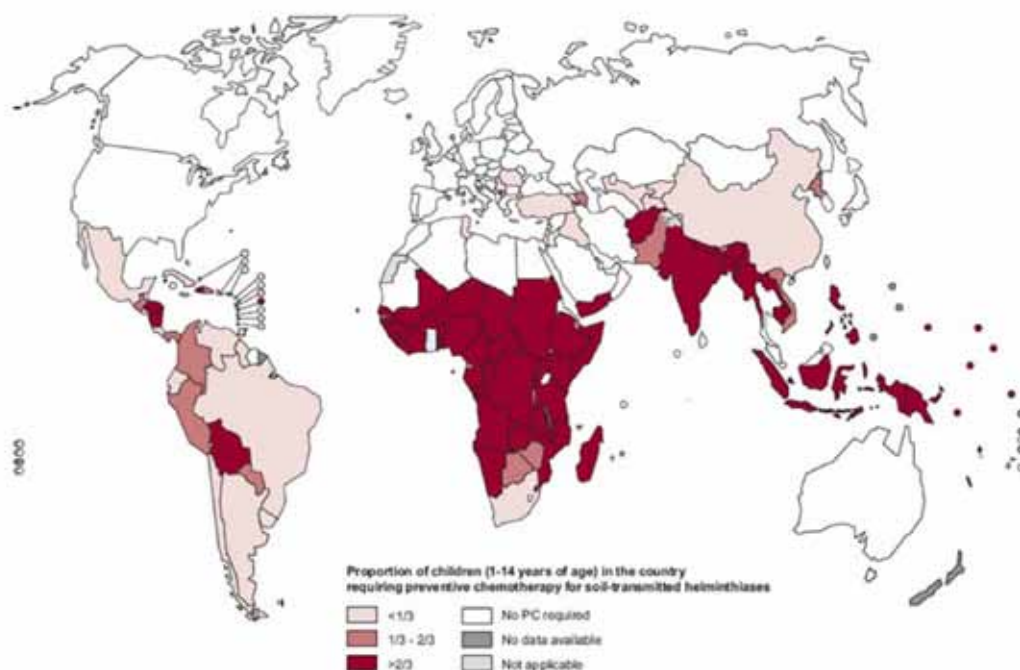


Figure 3.2. Global distribution of soil-transmitted helminth (STH) infections and proportion of children requiring preventive chemotherapy for STH infections in each country
(From World Health Organization. Helminth control in school-age children: a guide for managers of control programmes. 2nd ed. Geneva: World Health Organization; 2011.)

are estimated to have ascariasis, and about 2,000 die annually. The disease remains endemic in many countries of Southeast Asia, Africa, and Central and South America. Children ages 5 to 15 years have the highest intensities of infection with *Ascaris* compared with the other age groups. Children are particularly vulnerable since they are at risk of ingesting embryonated *Ascaris* eggs while playing in soil contaminated with human feces.

Worldwide estimates reveal that the highest number of cases of ascariasis is found in East Asia and the Pacific Islands, although *A. lumbricoides* is also known to be able to survive colder temperatures compared with *Trichuris* and hookworm. In many low and middle income countries like the Philippines, the prevalence may reach 80 to 90% in certain high risk groups like public elementary school children. Recent local sentinel surveys have

reported an overall prevalence of 27.7% among school-age children and 30.9% among preschool children. Prevalence rates are parallel with those of trichuriasis due to similar modes of infection and risk factors.

The level of transmission of *Ascaris* and other STH from soil to humans depends on socio-economic factors more than on physical factors. The main factors appear to be a high density of human population, involvement in agriculture (including use of night-soil as fertilizer), illiteracy, and poor sanitation. Poor health education on personal, family, and community hygiene are also important factors contributing to the transmission of *Ascaris*.

Prevention and Control

Surveillance and monitoring are important components of an STH Control Program. Baseline cumulative prevalence and prevalence

of heavy intensity infections should be compared with follow-up (pre-treatment) data (Table 3.1). The WHO recommends parasitologic monitoring involving the selection of 5 to 10 schools to represent a district or municipality. Stool samples from 50 school children from each school will be collected for examination

using Kato-Katz method. Monitoring is recommended every 2 years. Reinfection is usually observed four months post-treatment and full reinfection appears at 6 or 7 months after treatment; although in communities with poor environmental sanitation (Figure 3.3), reinfection may take place immediately after

Table 3.1. Core indicators of mass drug administration for soil-transmitted helminth infections

Indicator	Calculation (x 100%)	Target	Frequency
Treatment Coverage	<i>Numerator:</i> Population treated <i>Denominator:</i> Total population	DOH-IHCP: 85% among children 1–12 years of age, adolescent females, pregnant women, and treatment of other special population groups WHO: 75% among all preschool- (1–5 years) and school-age children (6–14 years)	In every round of treatment administration
Parasitologic evaluation	<p>Cumulative prevalence of STH infections in a population group: <i>Numerator:</i> # of individuals positive for any STH infection <i>Denominator:</i> # of individuals examined</p> <p>Heavy intensity infection rate of STH infections in a population group: <i>Numerator:</i> # of individuals with moderate and heavy intensity STH infection <i>Denominator:</i> # of individuals examined</p> <p>Prevalence rates per STH species in a population group: <i>Numerator:</i> # of individuals positive for a specific STH infection <i>Denominator:</i> # of individuals examined</p> <p>Proportion of heavy intensity infection per STH species in a population group: <i>Numerator:</i> # of individuals with moderate and heavy intensity <i>Ascaris</i>/<i>Trichuris</i>/hookworm infection <i>Denominator:</i> # of individuals positive for <i>Ascaris</i>/<i>Trichuris</i>/hookworm infection</p>	<p>Cumulative prevalence of STH infections: DOH-IHCP: <50% WHO: <20%</p> <p>Heavy intensity infection rates of STH infections: WHO: 0%</p>	Before the start of MDA and before next round of MDA in intervals of 2 to 3 years

Source:

(a) Department of Health, Administrative Order no. 2006-28: Strategic and operation framework for establishing Integrated Helminth Control Program (IHCP). 2006.

(b) World Health Organization. Helminth control in school-age children: A guide for managers of control programmes. 2nd ed. Geneva: World Health Organization; 2011.

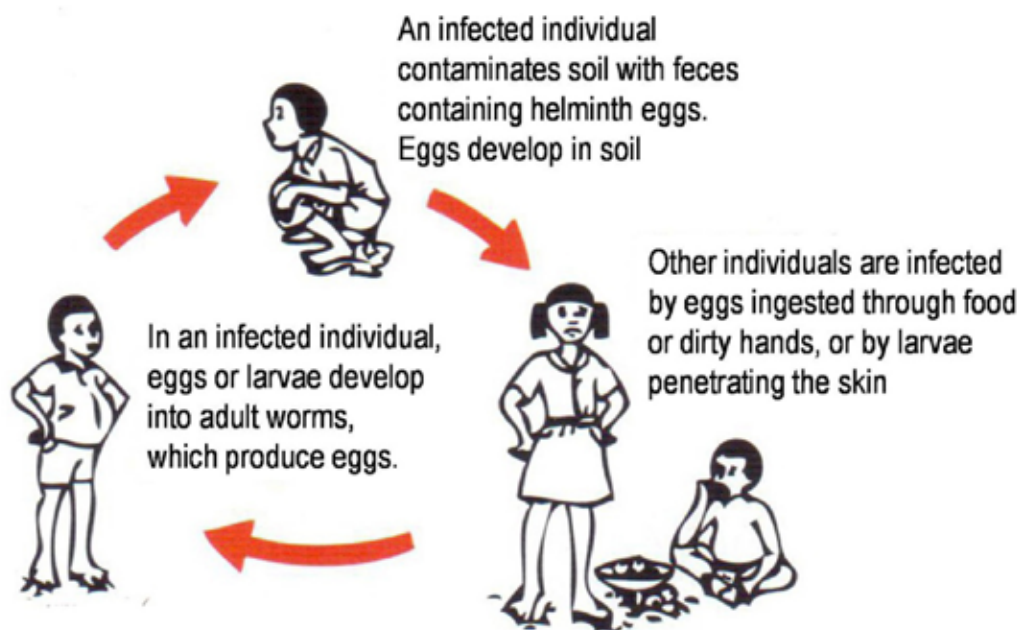


Figure 3.3. Schematic life cycle of soil-transmitted helminths
(From World Health Organization. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Geneva: World Health Organization; 2002. p. 145.)

deworming. Nutritional status and school performance may also be monitored alongside parasitologic parameters.

Prevention and control measures for *Ascaris* and other STH infections involve provision of safe water, environmental sanitation, hygiene education, and regular deworming, which are the components of the WASHED (water, sanitation, hygiene, education, deworming)

framework (Table 3.2) for the control of STH infections. When mass treatment is being undertaken, submission to the said intervention should be a goal of health education.

War on Worms (WOW) approach in Biñan, Laguna is a school-based, school teacher-assisted mass drug administration led by the Local Government Unit (LGU) which started in 1999. The approach was initially supported by

Table 3.2. The WASHED framework for a comprehensive control of soil-transmitted helminth infections

Water	<ul style="list-style-type: none">• Access to potable water• Drainage and disposal/re-use/recycling of household wastewater (also referred to as gray water)
Sanitation	<ul style="list-style-type: none">• Access to safe and sanitary sanitation facilities• Safe collection, storage, treatment, and disposal (feces and urine)• Management/re-use/recycling of solid waste
Hygiene Education	<ul style="list-style-type: none">• Appropriate information regarding prevention and treatment of STH infections• Dissemination of key messages to promote the following practices:<ul style="list-style-type: none">a. Safe water storageb. Safe handwashing and bathing practicesc. Safe treatment of foodstuffsd. Latrine usee. Use of footwear
Deworming	<ul style="list-style-type: none">• Regular mass drug administration (twice a year for school-age children)

Johnson & Johnson, Inc. (J&J) and eventually taken over by LGU and the Department of Education (DepEd) District of Biñan. Part of the WOW experience was that STH infections

do not reach the point of eradication due to implementation challenges and the limited practice of the WASHED strategies in the communities (Figure 3.4).

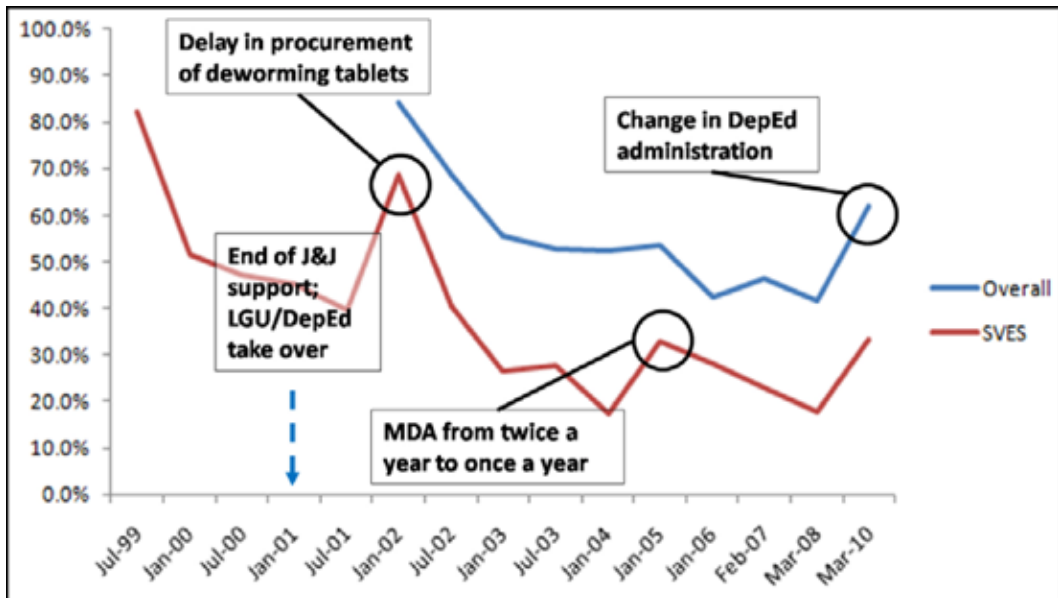


Figure 3.4. Comparison of cumulative prevalence in San Vicente Elementary School (SVES) and sentinel schools in Biñan, Laguna from 1999 to 2010 (Courtesy of Dr. Vicente Belizario, Jr.)

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Trichuris trichiura

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes

Trichuris trichiura or the whipworm is a soil-transmitted helminth, and is classified as holomyarian, based on the arrangement of somatic muscles in cross-section where the cells are small, numerous, and closely packed in a narrow zone.

Parasite Biology

The male worm (Plate 3.5a) measures 30 to 45 mm, slightly shorter than the female, which is 35 to 50 mm long. The female (Plate 3.5b) has a blunt posterior end, while the male has a coiled posterior with a single spicule and retractile sheath. The worms have an attenuated anterior three-fifths traversed by a narrow esophagus resembling a string of beads. The robust posterior two-fifths contain the intestine and a single set of reproductive organs. A female lays approximately 3,000 to 10,000 eggs per day.

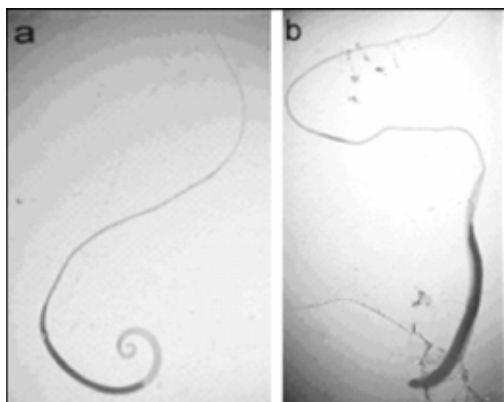


Plate 3.5. *Trichuris* male (a) and female (b)
(Courtesy of the Department of Parasitology,
UP-CPH)

The approximate measurements of the egg are 50 to 54 μm by 23 μm . It is lemon- or football-shaped with plug-like translucent polar prominences (Plate 3.6). The egg has a



Plate 3.6. *Trichuris* egg
(Courtesy of the Department of Parasitology,
UP-CPH)

yellowish outer and a transparent inner shell. Fertilized eggs are unsegmented at oviposition and embryonic development takes place outside the host when eggs are deposited in clayish soil. Compared with *Ascaris* eggs, *Trichuris* eggs in soil are more susceptible to desiccation.

Larvae are not usually described probably because soon after the embryonated eggs are ingested, the larvae escape and penetrate intestinal villi where they remain for 3 to 10 days. *Trichuris* worms inhabit the cecum and the colon. The worms secrete a pore-forming protein, called the TT47 that allows them to imbed their entire whip-like portion into the intestinal wall. After copulation, the female worm lays eggs, which are passed out with the feces and deposited in the soil. Under favorable conditions, the eggs develop and become embryonated within 2 to 3 weeks. If swallowed, the infective embryonated eggs go to the small intestine and undergo four larval stages to become adult worms. This process takes about 12 weeks (Figure 3.5). Unlike *Ascaris*, there is no heart-lung migration. Each female worm can produce about 60 million eggs over an average lifespan of 2 years.

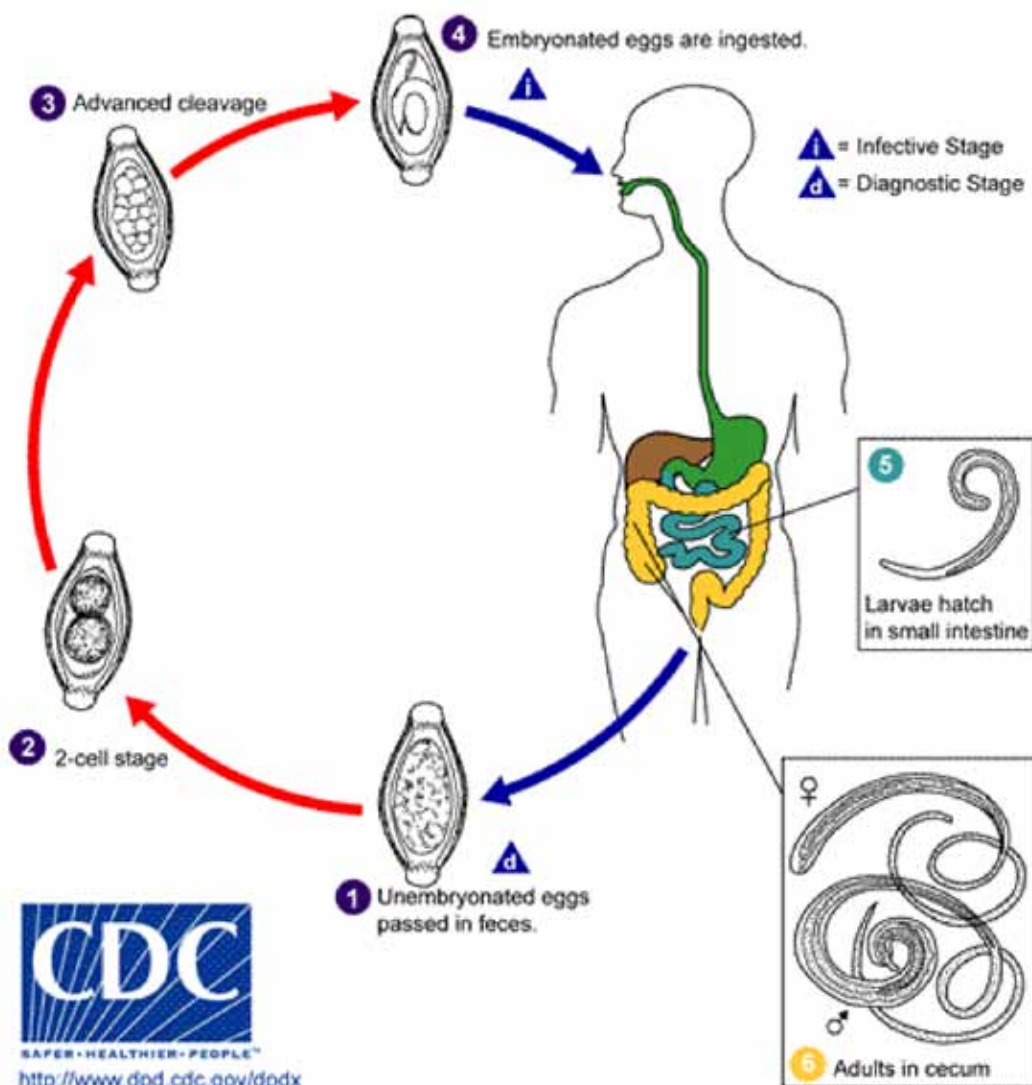


Figure 3.5. Life cycle of *Trichuris trichiura*
(Accessed from www.dpd.cdc.gov/dpdx)

Pathogenesis and Clinical Manifestations

The anterior portions of the worms, which are embedded in the mucosa, cause petechial hemorrhages, which may predispose to amebic dysentery, presumably because the ulcers provide a suitable site for tissue invasion by *E. histolytica*. The mucosa is hyperemic and edematous; enterorrhagia or intestinal bleeding

is common. The lumen of the appendix may be filled with worms, and consequent irritation and inflammation may lead to appendicitis or granuloma formation.

The intensity of infection is important in understanding the clinical picture. Infections with over 5,000 *T. trichiura* eggs per gram of feces are usually symptomatic. In patients with heavy intensity infection, the worms may be

found throughout the colon and rectum, and may result in *Trichuris* dysentery syndrome manifested by chronic dysentery and rectal prolapse (Plate 3.7). Such cases of heavy chronic trichuriasis are often marked by frequent blood-streaked diarrheal stools, abdominal pain and tenderness, nausea and vomiting, and weight loss. Anemia is strongly correlated to heavy intensity trichuriasis, and blood loss from such infections can range from 0.8 to 8.6 ml per day. Furthermore, infection with over 800 worms can result in anemia in children. On the other hand, light infections are moderately associated with anemia, although these infections are usually asymptomatic and the presence of the parasite may be discovered only in routine stool examinations. Trichuriasis has also been shown to result in poor appetite, wasting, stunting, as well as reduced intellectual and cognitive development in children.



Plate 3.7. Rectal prolapse in a 9-year old female seen at the Philippine General Hospital with heavy *Trichuris* infection
(Courtesy of Dr. Benjamin Cabrera)

The prognosis of trichuriasis is very good. Because there is no larval migration through the lungs as in *Ascaris* and hookworm infections, no lung pathology occurs.

Diagnosis

Clinical diagnosis is possible only in very heavy chronic *Trichuris* infection where the

patient suffers from frequent blood-streaked diarrhea, abdominal pain and tenderness, and rectal prolapse where adult worms attached to the rectal mucosa can be seen. In light infections where symptoms are absent, laboratory diagnosis is essential.

Laboratory diagnosis may be done by direct fecal smear (DFS) with a drop of saline. An alternative diagnostic technique is the Kato thick smear method that uses about 20 to 60 mg of stool sample. This method is highly recommended in the diagnosis of trichuriasis. The Kato-Katz technique is a quantitative method that employs egg counting to determine the intensity of helminth infection. This technique can be used to assess the efficacy of anthelmintic drugs in terms of cure rate (CR) and egg reduction rate (ERR). This technique can also be used for epidemiological surveys for the monitoring of a helminth control program. Both Kato thick and Kato-Katz techniques are simple and low-cost methods that have high sensitivity and specificity for the detection of *Trichuris* eggs, as well as eggs of other soil-transmitted helminths. A single Kato-Katz examination has a sensitivity and specificity for the detection of *Trichuris* of 91.4% and 94.4%, respectively.

The acid-ether and the formalin-ether/ethyl acetate concentration techniques can also be used for the diagnosis of trichuriasis. The FLOTAC technique has also been shown to be more sensitive in the diagnosis of trichuriasis compared with Kato-Katz and ether/ethyl acetate concentration techniques.

Treatment

The drug of choice in the treatment of trichuriasis is mebendazole given 100 mg twice a day for 3 days. Albendazole may be used as an alternative drug. Both are benzimidazole derivatives and are available as chewable tablets. Administration of mebendazole 500 mg once a day for 3 days has been shown to have the highest cure rate (71%) compared with albendazole 400

mg given once a day for 3 days (56%). For the purposes of preventive chemotherapy through mass drug administration, mebendazole is given as a 500 mg single dose, while albendazole is given as a 400 mg single dose. In recent local studies, it has been shown that albendazole in combination with ivermectin, a drug that is also used to treat filariasis, exhibited better cure and egg reduction rates than albendazole alone.

A contraindication for mebendazole and albendazole is hypersensitivity and early pregnancy (within the 1st trimester). Adverse effects of these two drugs are usually mild and transient and may present as headache, nausea, vomiting, gastrointestinal discomfort, and itchiness.

Deworming of children has been shown to contribute to improved motor and language development, as well as to reduced malnutrition. Nutritional status and intellectual development have also been shown to improve after deworming.

Epidemiology

Trichuriasis occurs in both temperate and tropical countries but is more widely distributed in warm, moist areas of the world. Approximately 604 to 795 million are infected globally. In tropical and subtropical regions, *Trichuris* is most prevalent in East Asia and Pacific Island regions, and least prevalent in the Middle East and North African regions. Among the different age groups, children 5 to 15 years of age are most frequently infected, and have the highest intensities of infection. In a recent sentinel survey in the Philippines, the prevalence of *Trichuris* ranged from 4.5 to 55.1% in preschool children, and from 8.1 to 57.9% in school-age children. Distribution of trichuriasis is similar to that of *A. lumbricoides*. Prevalence of co-infections with the two helminths is 19.1% in a recent sentinel survey.

Prevention and Control

Strategies for the prevention and control of *Trichuris* infection are similar to those for *Ascaris* infections. The WHO recommends biannual mass drug administration with mebendazole 500 mg or albendazole 400 mg among school-age children in communities where the prevalence of STH infections is $\geq 50\%$. Treatment of other high-risk groups such as preschool children, women of childbearing age, including pregnant women in the 2nd and 3rd trimesters as well as lactating women, adults in certain high-risk occupations should also be considered. On the other hand, once a year treatment is recommended in communities with STH prevalence $< 50\%$. Other strategies such as provision of safe water, environmental sanitation, and hygiene education are also important in STH control.

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Hookworms

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes, John Robert C. Medina

Necator americanus *Ancylostoma duodenale*

The hookworms that infect humans are *Necator americanus* and *Ancylostoma duodenale*, which are soil-transmitted helminths. They are blood-sucking nematodes that attach to the mucosa of the small intestines. They are most commonly found in tropical and subtropical countries where they occur as single or mixed infections.

Parasite Biology

All hookworms have the meromyarian type of somatic muscle with two to five cells arranged per dorsal or ventral half.

N. americanus adults are small, cylindrical, fusiform, grayish-white nematodes. Females (9–11 mm by 0.35 mm) are larger than males (5–9 mm by 0.30 mm). The posterior end of the male

has a broad, membranous caudal bursa with rib-like rays, which are used for copulation. The buccal capsule has a ventral pair of semilunar cutting plates (Plate 3.8a). The head is curved opposite to the curvature of the body, which is like a hook at the anterior end.

The adult *A. duodenale* is slightly larger than *N. americanus*. Each adult has single-paired male or female reproductive organs. Unlike the *N. americanus*, the head of the *A. duodenale* adult continues in the same direction as the curvature of the body. The buccal capsule has two pairs of curved ventral teeth (Plate 3.8b).

Rhabditiform larvae of *N. americanus* and *A. duodenale* are indistinguishable. They resemble those of *Strongyloides stercoralis*, but are somewhat larger, more attenuated posteriorly, and have a longer buccal cavity. The genital primordium is smaller in hookworms compared with *S. stercoralis*.

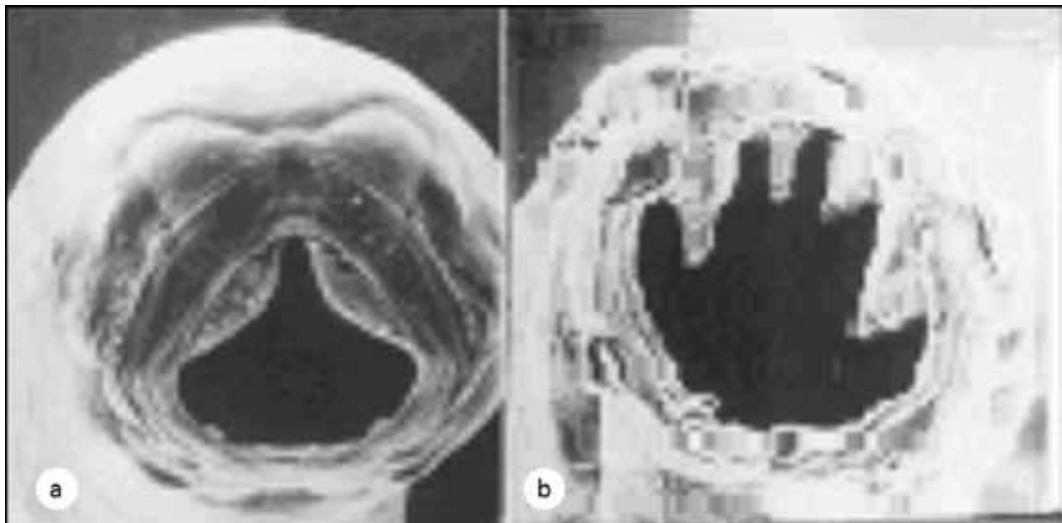


Plate 3.8. Buccal capsules of hookworms: *N. americanus* (a) and *A. duodenale* (b)
(Courtesy of Dr. Benjamin Cabrera)

The buccal spears of the *N. americanus* filariform larva (Plate 3.9) are conspicuous and parallel throughout their lengths. There are conspicuous transverse striations present on the sheath in the tail region. In contrast, the filariform larva of *A. duodenale* has inconspicuous buccal spears and transverse striations on the sheath in the tail region.



Plate 3.9. Hookworm filariform larvae
(Courtesy of the Department of Parasitology,
UP-CPH)

It is quite difficult to distinguish the eggs of *A. duodenale* from those of *N. americanus*. The eggs have bluntly rounded ends and a single thin transparent hyaline shell. They are unsegmented at oviposition, and are in the two- to eight-cell stage of division when passed out with fresh feces (Plate 3.10).

The hookworm life cycle (Figure 3.6) is direct and begins with the adult worms copulating while attached to the mucosa of the small intestines. Female worms oviposit into the intestinal lumen and the eggs are passed out with human feces. In the soil, the embryo within the egg develops rapidly and hatches after 1 to 2 days into the rhabditiform larva. After 7 to 10 days, the larva undergoes two stages of molting,

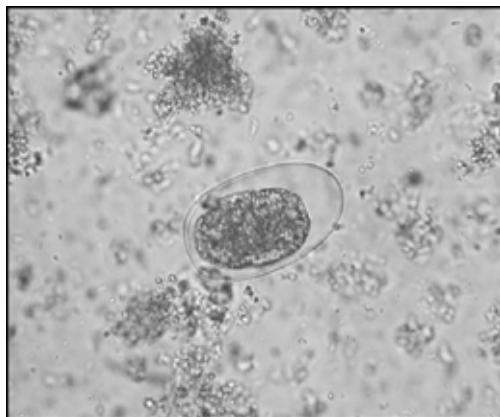


Plate 3.10. Hookworm egg
(Courtesy of the Department of Parasitology,
UP-CPH)

and transforms into the non-feeding filariform larva (L3), the infective stage of the parasite.

Filariform larvae penetrate the skin and enter venules. They migrate to the heart and lungs, and then into the alveoli. The larvae then ascend to the trachea and are finally swallowed, and passed down to the small intestine where the worms become sexually mature and the female will start laying eggs.

Pathogenesis and Clinical Manifestations

The pathology of hookworm infection involves: (a) the skin at the site of entry of the filariform larvae, (b) the lung during larval migration, and (c) the small intestine, the habitat of the adult worms.

Penetration of the filariform larvae through the skin produces maculopapular lesions and localized erythema. Itching is often severe, and it is known as “ground itch” or “dew itch,” as it is related to contact with soil, especially on a dewy morning. Itching, edema, erythema, and later papulovesicular eruptions can last for 2 weeks. If the larvae migrating through the lungs are abundant, bronchitis or pneumonitis may result. In the course of migration, these larvae produce minute hemorrhages with eosinophilic and leukocytic infiltration, but

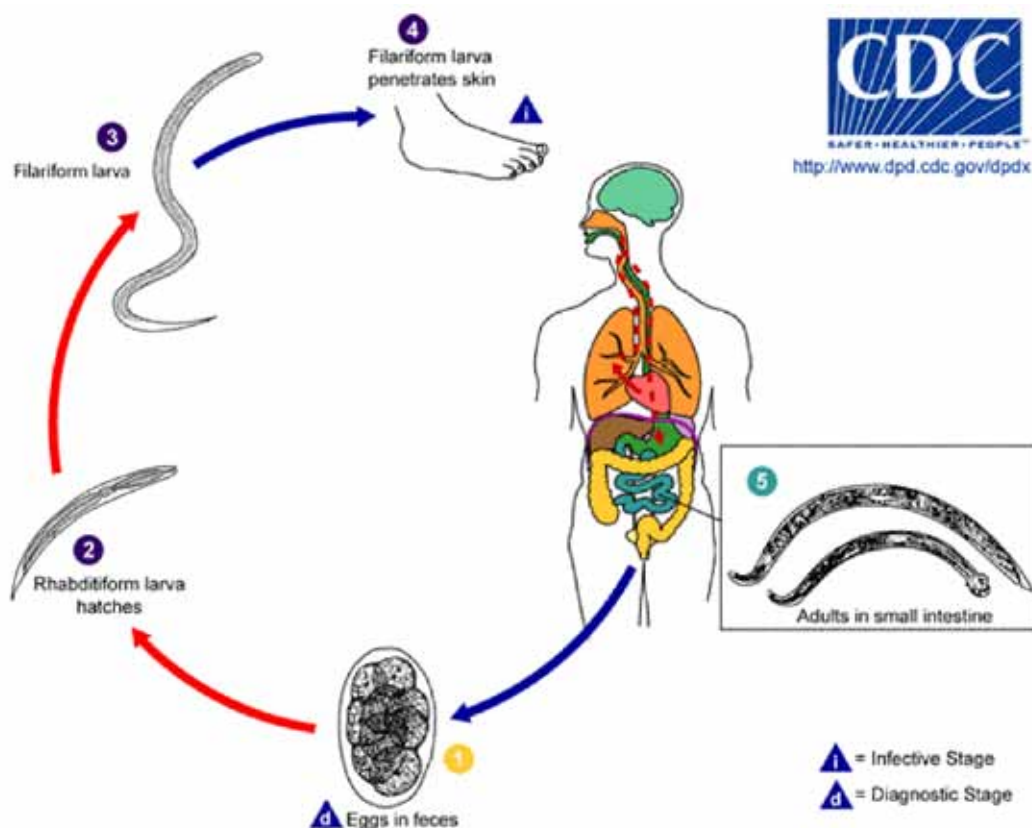


Figure 3.6. Life cycle of hookworms
(Accessed from www.dpd.cdc.gov/dpdx)

these manifestations seem to be rare in the tropics. In the stage of maturation of the worm in the intestine, there is abdominal pain, steatorrhea, or sometimes diarrhea with blood and mucus, as well as eosinophilia.

Hookworm infection is usually chronic, hence patients often show no acute symptoms. Studies have shown greater blood loss per worm per day in *A. duodenale* infection compared with *N. americanus* infection. Chronic moderate or heavy hookworm infection results in a progressive, secondary, microcytic, hypochromic anemia of the iron-deficient type, due primarily to continuous loss of blood.

Hypoalbuminemia is another manifestation of hookworm infection. There is low level of albumin due to combined loss of blood,

lymph, and protein. Other symptoms are exertional dyspnea, weakness, dizziness, and lassitude, while signs include rapid pulse, edema, and albuminuria. Unlike in ascariasis, the complications in hookworm infection are quite mild, and remedial measures are readily applied. In general, the prognosis of hookworm infection is good.

During the migration of the larva in the human body, the parasite continuously presents diverse immunogenic challenges to the host. Extensive humoral responses are produced against the larva and the adult hookworm, which share many antigens. Cellular immune response is primarily mediated by eosinophils, mast cells, and Th2 cells. Despite all of these, there has been no clear evidence that the

host develops perpetual immunity against hookworm infection; however, polyvalent IgE antibodies have been suggested to provide some protective roles.

Diagnosis

The clinical picture, though characteristic, is not pathognomonic to permit differentiation from other helminth infections. Final diagnosis depends on the identification of parasite ova in the feces. The following techniques are inexpensive and can be applied to both individual and mass screening:

1. Direct fecal smear is of value only when the infection is quite heavy. It may not detect the parasite in light infections (i.e., egg count of <400 eggs per gram feces).
2. The Kato thick or Kato-Katz method may increase detection rates since more stools are examined using these techniques. The latter technique may also provide quantitative diagnosis by determining the intensity of infection in terms of number of helminth eggs per gram of feces. The disadvantage of these methods is the rapid clearance of hookworm eggs after 30 to 60 minutes with the use of glycerine as a clearing agent.
3. Concentration methods like zinc sulfate centrifugal flotation and the formalin-ether/ethyl acetate concentration method use greater quantity of stool that may contribute to the increase in sensitivity. FLOTAC, which is also a centrifugal flotation method, has been shown to have a higher sensitivity for the diagnosis of soil-transmitted helminths compared with multiple examinations of Kato-Katz smears.
4. Culture methods like the Harada-Mori allow hatching of larvae from eggs on strips of filter paper with

one end immersed in water. Culture methods are recommended for species identification.

Molecular approaches, which include PCR-based detection of hookworm DNA in feces and enzyme-linked immunosorbent assay (ELISA) for the detection of secretory/excretory coproantigens, have also been developed.

Treatment

All diagnosed cases of hookworm infections should be treated; however, where the risk of reinfection is high, mass screening before treatment may be impractical. As with other soil-transmitted helminth infection control, the WHO recommends mass drug administration among school-age children at least once a year for communities with cumulative STH prevalence greater than or equal to 20%. Treatment of other high-risk groups such as preschool children, women of childbearing age, including pregnant women in the second and third trimesters and lactating women, should also be considered.

Albendazole, the drug of choice, is larvicidal and ovicidal against *N. americanus* and *A. duodenale*. It is given as a 400 mg single dose for adults and children over 2 years old. Chewable tablets or suspension preparations are available. Mebendazole for children and adults is given as a 500 mg single dose. These drugs are both benzimidazole derivatives that block the uptake of glucose by most intestinal and tissue nematodes. Adverse effects for both drugs are rare, and are usually mild and transient. These include epigastric pain, diarrhea, headache, and dizziness, among others.

Anemia and hypoproteinemia should also be addressed by giving iron supplementation and adequate diet.

In recent years, tolerance and resistance of human hookworms to these drugs had been reported in countries where regular deworming is the main control strategy. Studies had shown that the use of the recommended single dose

of the drugs led to low cure rate. Monitoring the efficacy of and drug resistance to these benzimidazole derivatives has not yet been done in the local setting. Baseline data are necessary for the evaluation and adjustment of the treatment regimen. Cure rates, egg reduction rates, and reinfection rates are important parameters in drug monitoring.

Epidemiology

About 576 to 740 million people in tropical and subtropical countries are estimated to be infected with either *A. duodenale* or *N. americanus*. Associated anemia causes at least 50,000 deaths annually.

Geographical distribution of the two human hookworms used to be relatively distinct. *A. duodenale* was prevalent in Europe and Southwestern Asia, while *N. americanus* was prevalent in tropical Africa and the Americas. But now, both species have become widely distributed throughout the tropics and subtropics, and rigid demarcations are no longer present.

In the Philippines, local studies on speciation of human hookworms revealed that out of 1,958 samples positive for hookworm in cultures, 97% were identified as *N. americanus*, 1% as *A. duodenale*, and 2% were mixed infections.

The local distribution of human hookworm infection is greater in agricultural areas. Farmers are prone to the infection because they work in rice fields and vegetable gardens, and they are not properly protected from contact with infective soil. In agricultural areas of Compostela Valley province, infection rates have been shown to be more than 50% in the late 1990s. Recent surveillance in sentinel sites in the Philippines revealed an overall prevalence of hookworm infection at 1.1% and 1.9% for preschool children and school children, respectively.

In other high-risk groups, the prevalence of hookworm infection remains relatively

high. Among pregnant women and adolescent females, the prevalence rates are 5.5% and 2.8%, respectively. A study among military and para-military personnel showed that 46.9% had the infection. In indigenous people communities in Davao del Norte, 13.6% of the school children were found to be infected. Among food handlers, 22.7% in Metro Manila and 14.8% in Cebu had hookworm infection.

Factors that contribute to the distribution and transmission of hookworms are: (a) suitability of the environment for eggs or larvae: damp, sandy or friable soil with decaying vegetation, and temperature of 24 to 32°C, (b) mode and extent of fecal pollution of the soil (through open defecation or the use of night soil as fertilizer), and (c) mode and extent of contact between infected soil and skin or mouth.

Whereas the method of human infection in necatoriasis is purely percutaneous, in ancylostomiasis, it is both percutaneous and through the oral route. The latter occurs upon eating raw vegetables contaminated with infective larvae and probably also through ingestion of raw or insufficiently cooked infected meat, although it is not clear whether infection through eating raw meat occurs in humans. *A. duodenale* may remain dormant in the intestines or in the muscles, resulting in a prolonged incubation period and creating problems in treatment. Transmammary transmission has also been reported.

In the Philippines, the first human case of *Ancylostoma ceylanicum* was reported in 1968 from a 53-year old woman from Ilocos Norte where 23 adult worms were collected. There are also animal hookworms like *Ancylostoma braziliense* (cat hookworm) and *Ancylostoma caninum* (dog hookworm) that can infect humans causing “creeping eruption,” also known as cutaneous larva migrans (CLM) (Plate 3.11).

Much of the necessary information about hookworm infection and the disease, i.e., morbidity and mortality rates, are still lacking



Plate 3.11. Cutaneous larva migrans
(Courtesy of Dr. Vicente Y. Belizario, Jr.)

in the Philippines. These are grounds for further local studies on the epidemiology of hookworm infection.

Prevention and Control

Regular mass drug administration in schools as part of the national control program had resulted to a decrease in the prevalence of soil-transmitted helminths among school children in a number of areas in the Philippines; however, coverage of deworming is limited to preschool- and school-age children, leaving other high-risk groups vulnerable.

In the Philippines, the WASHED approach is being advocated for a more comprehensive control of STH infections. This approach refers to improvement in access to clean water and sanitation, promotion of hygiene education, and regular deworming. Highlighting behavior change among the people and promotion of sustainable sanitation through community-led total sanitation may result in greater impact on helminth control. Open defecation should be discouraged and sanitary disposal of human feces, as well as wearing of shoes, slippers, and boots should be advised.

Because of the reported high rates of post-treatment reinfection, diminished efficacy of benzimidazole drugs, and concerns for drug resistance in many countries, development

of vaccines has been initiated by the Human Hookworm Vaccine Initiative—Sabin Vaccine Institute. In fact, a vaccine against a secretory antigen of hookworm had undergone a Phase I clinical trial on human volunteers. There were also on-going feasibility studies on the possibility of administering the vaccine along with anthelmintic drugs, Vitamin A, and micronutrients, as an intervention package for school children.

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Strongyloides stercoralis

Vicente Y. Belizario, Jr., Percy G. Balderia

This group of nematodes is characterized by free-living rhabditiform and parasitic filariform stages. *Strongyloides stercoralis* or threadworm is the only species of this genus which is naturally pathogenic to humans. Several species have been reported in mammals and in birds.

Parasite Biology

The parasitic or filariform female is 2.2 mm by 0.04 mm, colorless, semi-transparent, with a finely striated cuticle. It has a slender tapering anterior end and a short conical pointed tail. The short buccal cavity has four indistinct lips. The long slender esophagus extends to the anterior fourth of the body, and the intestine is continuous to the subterminal anus. The vulva is located one-third the length of the body from the posterior end. The uteri contain a single file of 8 to 12 thin-shelled, transparent, segmented ova, 50 to 58 μm by 30 to 34 μm .

The free-living female (Plate 3.12) measures 1 mm by 0.06 mm and is smaller

than the parasitic female. It has a muscular double-bulbed esophagus, and the intestine is a straight cylindrical tube. The free-living male, measuring 0.7 mm by 0.04 mm, is smaller than the female, and has a ventrally curved tail, two copulatory spicules, a gubernaculum, but no caudal alae. Parasitic males have not been reliably identified.

The rhabditiform larva measures 225 μm by 16 μm . It has an elongated esophagus with a pyriform posterior bulb. This species differs from the hookworm in being slightly smaller and less attenuated posteriorly. It also has a shorter buccal capsule and a larger genital primordium.

The infective filariform larva is non-feeding, slender, and about 550 μm in length. It is similar to the hookworm filariform larva but is usually smaller, with a distinct cleft at the tip of the tail.

Eggs have a clear thin shell and are similar to those of hookworms except that they measure only about 50 to 58 μm by 30 to 34 μm .

Free-living forms of *Strongyloides* are found in the soil. The female worm lays embryonated eggs, which develop into rhabditiform larvae after a few hours. These larvae feed on organic matter and transform into free-living adults. When conditions in the soil become unfavorable, rhabditiform larvae develop into filariform larvae, which are infective to humans.

The parasitic life cycle begins when filariform larvae infect humans through the skin. The parasites enter the circulation, pass through the lungs, and migrate to the larynx where they are subsequently swallowed. Larvae develop into adults in about a month while in the duodenum. Females generally reproduce by parthenogenesis. They invade the intestinal mucosa where they deposit their eggs. Eggs



Plate 3.12. *Strongyloides stercoralis* rhabditiform larva (Courtesy of the Department of Parasitology, UP-CPH)

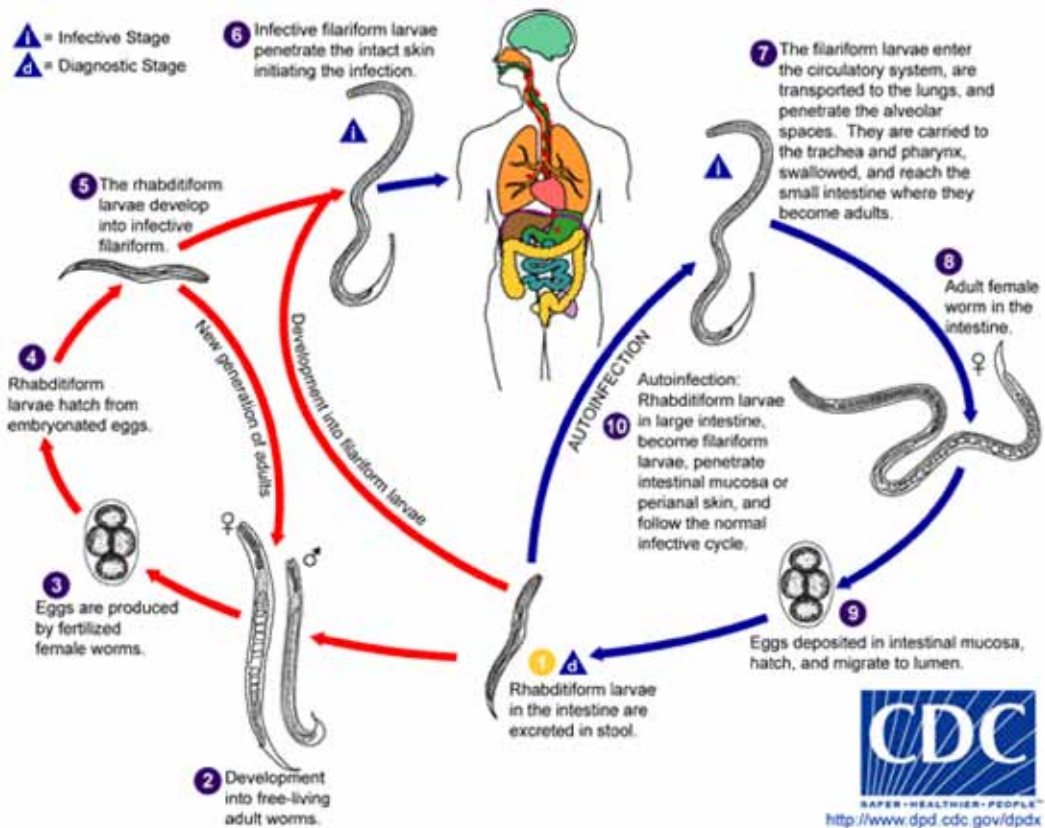


Figure 3.7. Life cycle of *Strongyloides stercoralis*
(Accessed from www.dpd.cdc.gov/dpdx)

hatch into rhabditiform larvae, migrate into the lumen, and pass out in the feces.

Autoinfection occurs when rhabditiform larvae pass down the large intestine and develop into filariform larvae. Being the infective stage, these filariform larvae may invade the mucosa and enter the circulation to start another parasitic cycle without leaving the body of the host (Figure 3.7).

Pathogenesis and Clinical Manifestations

There are three phases of acute infection in strongyloidiasis: (a) invasion of the skin by filariform larvae, (b) migration of larvae through the body, and (c) penetration of the intestinal mucosa by adult female worms. The migration of larvae through the body and penetration of

intestinal mucosa by adult females may occur simultaneously, particularly in hyperinfection.

In the first phase of acute infection, larval invasion of the skin produces erythema, and pruritic elevated hemorrhagic papules. During the larval migration phase, the lungs are destroyed causing lobar pneumonia with hemorrhage. Cough and tracheal irritation may also occur, mimicking bronchitis. In the third phase, adult female worms may be found in the intestinal mucosa from the pylorus to the rectum, but the greatest numbers are found in the duodenal and upper jejunal regions.

Light infection does not cause intestinal symptoms. Moderate infection causes diarrhea alternating with constipation. Heavy infection produces intractable, painless, intermittent

diarrhea (Cochin China diarrhea) characterized by numerous episodes of watery and bloody stools. Hyperinfection is a syndrome of accelerated autoinfection which usually, but not invariably, occurs in the immunocompromised. It manifests with exacerbation of gastrointestinal and pulmonary symptoms and increased numbers of larvae in the stool and/or sputum.

Chronic strongyloidiasis is often asymptomatic. However, intermittent vomiting, diarrhea, constipation, and borborygmi may be observed. Anal pruritus, urticaria, and larva currens rashes are also common. Recurrent asthma and nephritic syndrome have also been reported in cases of chronic infection with *S. stercoralis*.

Complications include edema, emaciation, loss of appetite, anemia, lobar pneumonia, ileus, intestinal obstruction, gastrointestinal bleeding, and malabsorption leading to cachexia.

Prognosis is good in light infections, but moderate and heavy infections have high mortality rates due to the massive invasion of tissues by adults and larvae. Disseminated infection occurs among patients with cancer, malnutrition, HIV/AIDS, HTLV-1, or those using immunosuppressive drugs after organ transplantation.

Diagnosis

The finding of unexplained eosinophilia in a patient may be a clue pointing to strongyloidiasis. The application of repeated concentration techniques, like the Baermann funnel gauze method, usually leads to detection of the infection. Harada-Mori culture is considered one of the most successful methods in parasite identification. At present, using the nutrient agar plates is also recommended. Other laboratory methods that can be done are Beale's string test, duodenal aspiration, and small bowel biopsy. In disseminated strongyloidiasis, larvae may be found in sputum or urine. Serology may not be useful in filariasis endemic areas since there are cross-reactions between *Strongyloides* and filarial worm antigens.

The culture technique is practical, low-cost, and suited for mass screening as well as individual diagnosis. The modified Harada-Mori culture method makes use of polyethylene plastic bags or tubes instead of glass tubes. Plastic bags and tubes are unbreakable, lighter to transport, and do not occupy much space. These are therefore recommended for use in the field. On the other hand, the main advantage of serologic testing is the rapidity and ease of performance of the procedure.

Treatment

All infected individuals should be treated. Treatment was previously based on albendazole or thiabendazole. However, recent studies show that ivermectin also provides the best results in chronic uncomplicated strongyloidiasis with regard to efficacy and tolerability. Higher doses given for longer periods may be necessary. *Strongyloides stercoralis* is quite sensitive to the ovicidal and larvicidal actions of the drugs. Albendazole, thiabendazole, and ivermectin have been used to treat hyperinfection or disseminated disease singly or in combination, but data are limited to case reports or case series.

Albendazole and thiabendazole are contraindicated in pregnant women and in those with known hypersensitivity to the drugs. Thiabendazole may give rise to dizziness, gastrointestinal irritation, drowsiness, pruritus, and headache lasting for several hours. Adverse reactions with albendazole are transient gastrointestinal discomfort and headache.

Egg reduction rate cannot be determined because eggs are not passed out in the feces but are oviposited in the intestine and other tissues of the host. Reinfection rate is difficult to calculate because of autoinfection.

Epidemiology

Strongyloides stercoralis is found throughout the world and follows a distribution pattern similar to hookworm in the tropics and subtropics, as well as in Europe and the USA. Some 50 to 100 million people are estimated

to be infected with this parasite. *Strongyloides* is a soil-transmitted helminth.

In the Philippines, strongyloidiasis is relatively rare. Local data on the prevalence of *Strongyloides stercoralis* reveal that out of 4,208 stools examined using Harada-Mori culture, only 50 samples or 1.2% were found positive for the worm. If all studies on prevalence were included, out of 294,176 stools examined, only 148 or 0.05% were found positive. Prevalence rates have been described to fluctuate between 0 to 2.3%, depending on the area selected. This infection is more frequent in male children 7 to 14 years old, than among females and adults.

Infection and disease rates as well as morbidity and mortality figures are not well documented. The factors that affect transmission include poor sanitation and indiscriminate disposal of human feces that may contain *Strongyloides* larvae. Autoinfection explains how some people remain infected for more than 30 years even after leaving the endemic area. This phenomenon has been seen in American veterans who returned from the Korean and Vietnam wars.

Prevention and Control

Prevention and control measures for this disease are similar to those for hookworm infection. Both worms use the soil for further development and maintain their endemicity in areas where environmental sanitation is poor and human feces is deposited indiscriminately in the soil by infected people. Infection is acquired by individuals who usually walk barefoot. There is a need to provide health education on personal, family and community hygiene to change behavior and practices. Infected individuals should be treated in order to prevent morbidity

and mortality. People with cancer, debilitating diseases like pulmonary tuberculosis, and malnutrition, and those about to undergo organ transplantation should be cleared of *Strongyloides* infection. This important step is taken to prevent the occurrence of disseminated strongyloidiasis, which is almost always fatal because larvae invade vital organs.

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Enterobius vermicularis

Vicente Y. Belizario, Jr., Percy G. Balderia

Enterobius vermicularis or human pinworm causes enterobiasis or oxyuriasis. The infection is typically characterized by perianal itching or pruritus ani. Although not a usual cause of significant morbidity or mortality, migrating worms may go beyond the perianal region and can occasionally cause complications in ectopic areas.

This intestinal nematode is classified as meromyarian, based on the arrangement of the somatic muscles where there are two to five cells per dorsal or ventral half.

The human pinworm is the most common helminth parasite identified in temperate regions, where environmental sanitation is in place. Less attention is given to pinworm infection in tropical areas, likely due to the presence of other, more clinically significant parasites.

Parasite Biology

Adult worms have cuticular alar expansions (Plate 3.13) at the anterior end and a prominent

posterior esophageal bulb. The small adult female worm measures 8 to 13 mm by 0.4 mm and has a long pointed tail. The uteri of gravid females are distended with eggs. The male, measuring 2 to 5 mm by 0.1 to 0.2 mm has a curved tail and a single spicule. Males are rarely seen because they usually die after copulation.

The rhabditiform larva, measuring 140 to 150 μm by 10 μm , has the characteristic esophageal bulb, but has no cuticular expansion on the anterior end.

Eggs (Plate 3.14) are asymmetrical, with one side flattened and the other side convex, and range from 50 to 60 μm by 20 to 30 μm in size averaging 55 by 36 μm . The translucent shell consists of an outer triple albuminous covering for mechanical protection and an inner embryonic lipoidal membrane for chemical protection. Inside the egg is a tadpole like embryo that becomes fully mature outside the host within 4 to 6 hours.

Adult worms are found in the cecum and adjacent portions of the small and large

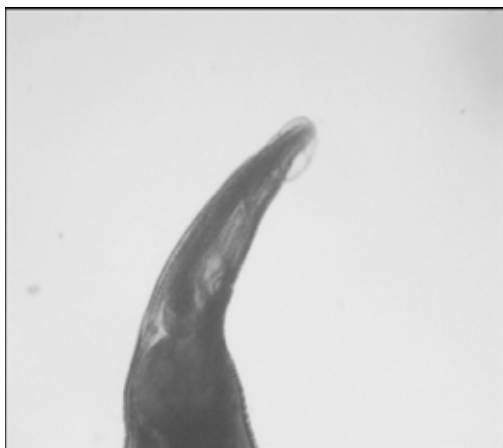


Plate 3.13. *Enterobius* cephalic alae
(Courtesy of the Department of Parasitology,
UP-CPH)



Plate 3.14. D-shaped eggs of *Enterobius vermicularis*
(Courtesy of the Department of
Parasitology, UP-CPH)

intestines. Gravid female worms migrate down the intestinal tract and exit through the anus to deposit eggs on the perianal skin. Adult female worms migrate to the perianal area, usually in the evening hours. A single female lays from 4,672 to 16,888 eggs per day with an average of 11,105 eggs. After egg deposition, the female

usually dies. Eggs on the perianal region become fully embryonated within 6 hours. When ingested, eggs containing the 3rd stage larvae hatch in the duodenum, pass down the small intestines to the cecum, and develop into adults (Figure 3.8). Eggs are resistant to disinfectants but succumb to dehydration in dry air within

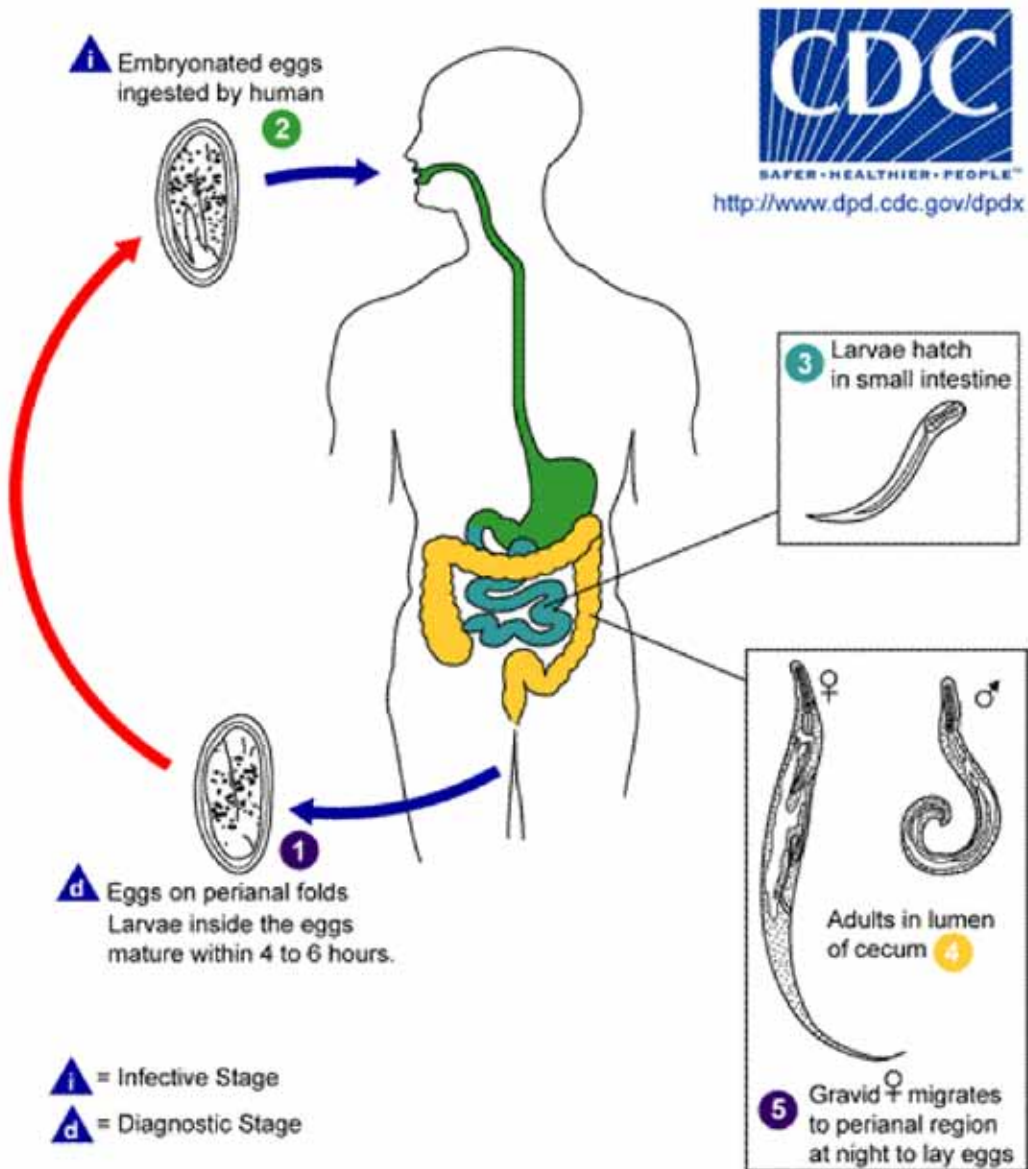


Figure 3.8. Life cycle of *Enterobius vermicularis*
(Accessed from www.dpd.cdc.gov/dpdx)

a day. However, in moist conditions, these eggs can remain viable for up to 13 days. The eggs remain viable longest under conditions of fairly high humidity and moderate temperature. The eggs may survive for some days in dry dust, and airborne eggs can infect persons at a distance via inhalation.

Pathogenesis and Clinical Manifestations

Enterobius vermicularis is a relatively innocuous parasite and rarely produces any serious lesions. Mild catarrhal inflammation of the intestinal mucosa may result from the attachment of the worms. Mechanical irritation and secondary bacterial invasion may lead to inflammation of the deeper layers of the intestines. Invasion of the appendix is not unusual, but whether this invasion is a significant cause of appendicitis is not known. Migration of egg-laying females to the anus causes irritation of the perineal region. Intense itching leads to scratching, and may give rise to secondary bacterial infection. Children infected with this parasite may suffer from insomnia due to the pruritus. Other signs of infection are poor appetite, weight loss, irritability, grinding of teeth, and abdominal pain.

Complications such as appendicitis, vaginitis, endometritis, salpingitis, and peritonitis are all due to aberrant adult worm migration. Entry into the peritoneal cavity via the female reproductive system may result in the formation of granuloma around eggs or worms. Pinworms or their eggs have occasionally been reported from other ectopic sites such as the liver and lung.

The prognosis of enterobiasis or oxyuriasis is good. This parasitic disease is extremely contagious and can easily spread among members of a family or in institutions. Hence, it has been described as a familial or a group disease.

Diagnosis

Enterobiasis should be suspected in children and adults who show perianal itching

relieved only by vigorous scratching. Diagnosis is confirmed by finding adult worms or eggs on microscopic examinations. Adult worms may be seen in the feces or in the perianal region. Eggs are found in the feces in only about 5% of infected persons. The method of laboratory diagnosis is the Graham's scotch adhesive tape swab (perianal cellulose tape swab), which gives the highest percentage of positive results, and the greatest number of eggs seen. This low-cost diagnostic method is easy to perform and is very sensitive and specific.

Treatment

The drugs of choice are mebendazole 100 mg PO single dose or albendazole 400 mg PO single dose. Pyrantel pamoate 11 mg/kg base PO single dose (max. of 1 g) is considered a secondary drug of choice. *E. vermicularis* is quite susceptible to these drugs, with reported cure rates of over 90%. Moreover, since family members are usually infected, treatment of the entire household is recommended. Cure can only be considered after seven perianal smears, on consecutive days using scotch-tape swab method, are all found to be negative. The egg reduction rate is difficult to determine because eggs are collected from the perianal area instead of from the feces using Kato-Katz. Mebendazole, albendazole, and pyrantel are contraindicated in individuals with known hypersensitivity. Adverse effects of these drugs include mild, transient gastrointestinal disturbance, and headache.

Epidemiology

Enterobiasis occurs in both temperate and tropical regions of the world, and has a high prevalence in both developed and developing countries. It is the only intestinal nematode infection that cannot be controlled through sanitary disposal of human feces, because the eggs are deposited in the perianal region instead of the intestinal lumen. Eggs usually contaminate underwear and beddings. The route of infection is through the mouth, the

respiratory system (by inhalation of dust containing *Enterobius* eggs), and through the anus (wherein the hatched larvae enter the anus and cause reinfection when they go back into the large intestine). Risk factors for infection include overcrowding, thumb-sucking, nail-biting, and lack of parental knowledge on pinworms.

There are around 208.8 million infected persons in the world, with 18 million in Canada and the United States of America. Prevalence is 12 to 41% in Washington, D.C. In the Philippines, prevalence levels have been found to be 29% among schoolchildren from exclusive private schools, and 56% among those from public schools. Locally, prevalence is consistently higher in females (16%) compared to males (9%). Eggs were found in nail clippings of school children.

Local data on infection and disease rates, as well as morbidity and mortality figures are inadequate.

Prevention and Control

Personal cleanliness and personal hygiene are essential. Fingernails should be cut short and hand washing should be done after using the toilet, as well as before and after meals. The use of showers rather than bathtubs is suggested, and infected persons should sleep alone until adequately treated. Underwear, night clothes, blankets, and bed sheets should be handled with care and washed in hot soapy water. Vacuum cleaning around beds and contaminated areas will be useful. Being a familial disease, chemotherapy of the entire family is recommended, and will help in the control of the disease.

The implementation of mass drug administration targeting soil-transmitted helminthiasis is expected to have an impact on the prevalence of enterobiasis as well. Control efforts in elementary schools provide

opportunities for health education of teachers and school children regarding measures on control and prevention of intestinal helminth infections, including pinworm infections.

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Capillaria philippinensis

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes

Capillaria philippinensis is one of four *Capillaria* species that are known to infect humans. Human infection with *C. philippinensis* was first reported by Chitwood et al. in 1963 in a 29 year old male from Northern Luzon. Intestinal capillariasis, a zoonotic disease, is characterized by abdominal pain, chronic diarrhea, and gurgling stomach. The disease may also be associated with protein-losing enteropathy, electrolyte imbalance, and

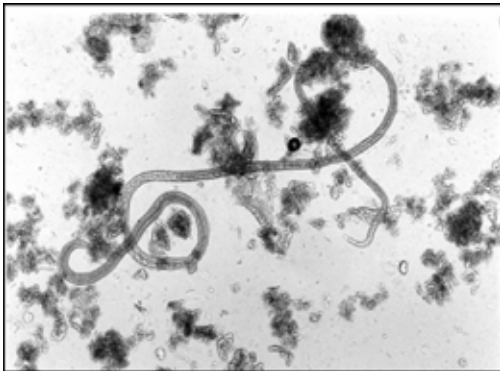


Plate 3.15. Male *Capillaria philippinensis*
(Courtesy of Dr. John Cross)

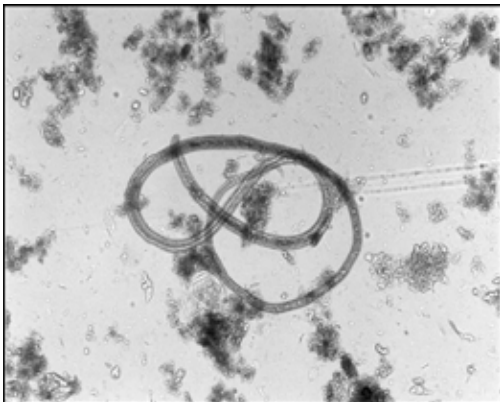


Plate 3.16. Female *Capillaria philippinensis*
(Courtesy of Dr. John Cross)

intestinal malabsorption. Severe disease can result in death. Fish-eating birds are the natural hosts of the nematode.

Parasite Biology

Capillaria philippinensis is a nematode from the superfamily Trichinelloidea, to which *Trichuris* and *Trichinella* belong. The parasites in this superfamily characteristically have a thin filamentous anterior end and a slightly thicker and shorter posterior end. The male worms (Plate 3.15) are about 1.5 to 3.9 mm in length, while females (Plate 3.16) are 2.3 to 5.3 mm long. The male spicule is 230 to 300 μm long and has an unspined sheath. The esophagus has rows of secretory cells called stichocytes, and the entire esophageal structure is called a stichosome. The anus is subterminal, and the vulva in females is located at the junction of anterior and middle thirds.

Female worms produce characteristic eggs, which are peanut-shaped with striated shells and flattened bipolar plugs (Plate 3.17). These eggs, which measure 36 to 45 μm by 20 μm , are passed in the feces and embryonate in

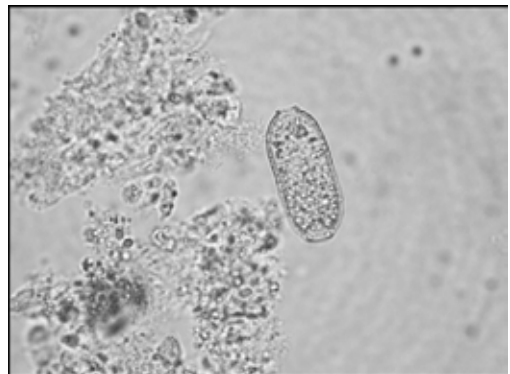


Plate 3.17. *Capillaria philippinensis* egg
(Courtesy of the Department of Parasitology,
UP-CPH)

the soil or water. They must reach the water in order to be ingested by small species of freshwater or brackish water fish (Figure 3.9).

The eggs hatch in the intestines of the fish and grow into the infective larvae. When the fish is eaten uncooked, the larvae escape from the

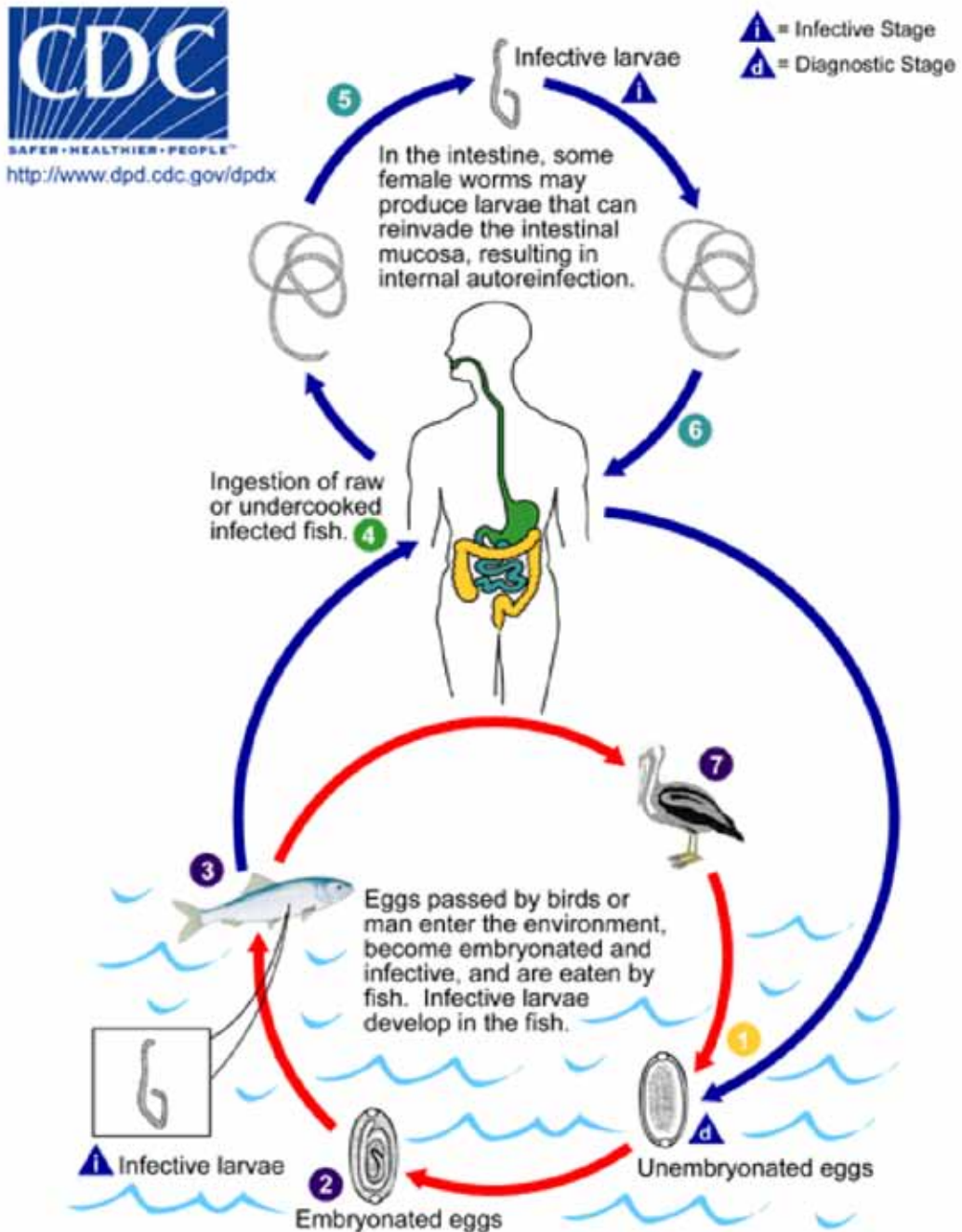


Figure 3.9. Life cycle of *Capillaria philippinensis*
(Accessed from www.dpd.cdc.gov/dpdx)

fish intestines and develop into adult worms in human intestines.

The first generation of female worms produces larvae to build up the population. Subsequent generations predominantly produce eggs, although there are always a few female worms that produce both larvae and eggs, or larvae only. Some of these larvae are retained in the gut lumen and develop into adults. This leads to hyperinfection and autoinfection, which result in the production of very large numbers of worms. In one autopsy, as many as 200,000 worms were recovered from one liter of bowel fluid.

Fish-eating birds are believed to be the natural hosts of *C. philippinensis*, and humans are considered incidental hosts.

Pathogenesis and Clinical Manifestations

Persons with *C. philippinensis* usually have abdominal pain and borborygmi. Patients initially experience intermittent diarrhea, which progresses to passing out 8 to 10 voluminous stools per day. After a few weeks, there is noticeable weight loss, malaise, anorexia, vomiting, and edema. Laboratory findings show severe protein-losing enteropathy and hypoalbuminemia; malabsorption of fats and sugars; decreased excretion of xylose; low serum potassium, sodium and calcium; and high levels of immunoglobulin E. If the disease is not treated soon after the symptoms occur, severe manifestations of the disease develop with a potentially fatal outcome.

The large number of worms that develop in humans is responsible for the severe pathology. The parasites do not invade intestinal tissue, but they are responsible for micro-ulcers in the epithelium, and the compressive degeneration and mechanical compression of cells. Homogeneous material is seen at the anterior end of the worm by electron microscopy. The ulcerative and degenerative lesions in the intestinal mucosa may account for malabsorption of fluid, protein, and electrolytes.

Endoscopic finding may reveal non-specific segmental erythematous inflammation in the small bowel with superficial erosions with exudation.

Histologically, the intestines also show flattened and denuded villi, and dilated mucosal glands. The lamina propria is infiltrated with plasma cells, lymphocytes, macrophages, and neutrophils.

Diagnosis

Diagnosis is based on finding characteristic eggs in the feces by direct smear or wet mount, as well as by stool concentration methods. There may also be various larval stages of the parasites, as well as adult worms in the feces. The uterus of the female worms may contain developing eggs and sometimes larvae (Plate 3.18). The parasites can also be recovered from the small intestines by duodenal aspiration.



Plate 3.18. *Capillaria philippinensis* second stage larva from the feces of a person with intestinal capillariasis (Courtesy of Dr. John Cross)

A study done in Egypt demonstrated high specificity of sandwich enzyme-linked immunosorbent assay (ELISA) in the detection of coproantigen prepared from stool samples of patients with capillariasis. This technique did not show cross-reaction with coproantigen from patients with *Fasciola gigantica* and *Schistosoma mansoni*. Another study demonstrated cross-reaction of capillariasis patient antibodies with *Trichinella spiralis* antigen in immunoblot assay, suggesting the prospective use of *T.*

spiralis antigen for the immunodiagnosis of capillariasis. ELISA using *T. spiralis* antigen has been tested and shown to have a sensitivity of 100% in the diagnosis of capillariasis (43 positive cases) and a specificity of 100% (57 negative cases).

Treatment

In severe cases with electrolyte and protein loss, patients should be given electrolyte replacement and a high protein diet (Plate 3.19). Anthelmintic drugs should also be given. The drug of choice for the treatment of intestinal capillariasis is mebendazole, 200 mg twice a day for 20 days. Alternatively, albendazole 400 mg may be given once daily for 10 days. Relapses may occur if the treatment regimen is not followed and completed.



Plate 3.19. 31-year old female with intestinal capillariasis before treatment (left) and 1 year after treatment (right) (Courtesy of Dr. Vicente Belizario, Jr.)

Epidemiology

Intestinal capillariasis was first recorded in Northern Luzon in the Philippines. In 1966, an epidemic in Pudoc West, Tagudin, Ilocos Sur was reported, that spread to neighboring

towns and resulted in more than 1,000 cases and 77 deaths. Cases of human capillariasis have been subsequently reported in Thailand, Iran, Japan, Indonesia, United Arab Emirates, South Korea, India, Taiwan, Egypt, and Lao People's Democratic Republic. A review of data from local hospitals throughout Taiwan from 1983 to 2003 revealed a total of 30 capillariasis cases, 21 of whom were from two major Taiwanese aboriginal tribes.

In the Philippines, nearly 2,000 cases have been documented from the Northern Luzon provinces from 1967 to 1990. Cases have also been documented in Zambales and Southern Leyte. Infections are acquired by eating uncooked small freshwater/brackish water fish. Ilocano people enjoy eating *bagsit* and other fishes found in the lagoons. In Monkayo, Compostela Valley Province, an outbreak described as a "mystery disease" in 1998 resulted in the death of villagers due to misdiagnosis. Intestinal capillariasis was diagnosed in 17% of the cases presenting with chronic diarrhea. A more recently described endemic area in the Philippines involved Zamboanga del Norte, where more than 70 deaths were recorded and 4.9% of those examined in a parasitologic survey were confirmed to have capillariasis. A few cases have also been confirmed in Zamboanga del Sur, Agusan del Sur, and Misamis Occidental.

Prevention and Control

It is believed that the 1967 to 1968 Philippine epidemic was due to washing of fecally contaminated bed sheets in lagoons in the Tagudin area of Ilocos Sur. Efforts to improve sanitation and health educational programs to prevent indiscriminate disposal of human waste and to discourage eating raw fish are important in controlling the spread of infection (Plate 3.20). Capacity building for health personnel in the field, including laboratory staff, for early and accurate diagnosis and treatment is important in preventing mortality. Health education can also help improve patient health-seeking behaviors.



Plate 3.20. Proper excreta disposal is important for prevention and control of intestinal helminthiasis including capillariasis (Courtesy of Dr. Vicente Belizario, Jr.)

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Tissue Nematodes

Vicente Y. Belizario, Jr., Timothy M. Ting

Lymphatic Filariasis

Wuchereria bancrofti *Brugia malayi*

There are eight known species of filarial nematodes that use humans as their definitive host. These are subdivided into three groups based on the anatomic location from which they cause pathology: subcutaneous, serous cavity, and lymphatic filariasis. *Mansonella* causes serous cavity filariasis in the abdomen. Filarial worms that live in the subcutaneous fat under the skin include *Loa loa* (African eye worm), *Mansonella streptocerca*, and *Onchocerca volvulus*. Lymphatic filariasis is caused by *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. With adults that become lodged in the lymphatic system, these worms cause lymphedema, lymphangitis, and in chronic cases, elephantiasis. Disease is transmitted by blood-feeding arthropod vectors, mainly mosquitoes and black flies.

Lymphatic filariasis (LF) is one of the most debilitating diseases plaguing many tropical countries. Next to psychiatric illness, LF is the second leading cause of permanent and long-term disability, affecting both physical and psychological aspects of the victim. The social stigma and associated economic consequences result in a poor quality of life to the afflicted. The two most common mosquito-borne causative agents of LF are *Wuchereria bancrofti* or Bancroft's filarial worm, which is the causative agent of Bancroftian filariasis; and *Brugia malayi* or the Malayan filarial worm, which causes Malayan filariasis.

Parasite Biology

Adult *Wuchereria* worms are creamy white, long, and filiform in shape. The male worm measures 20 to 40 mm in length, while the female measures 80 to 100 mm. Microfilariae in fresh specimens appear as minute snake-like organisms constantly moving among the red blood cells. A microfilaria measures 270 to 290 μm and is enclosed in a hyaline sheath which is much longer than the microfilaria itself (Figure 3.22). When stained, the central axis shows dark-staining nuclei, which serve as an important identifying feature. The column of nuclei is arranged in two or three rows and is distinctly conspicuous. Microfilariae have several curvatures and a graceful appearance.

The *Brugia* male measures 13 to 23 mm in length while the female measures 43 to 55 mm. Adult females of *B. malayi* and *W. bancrofti* are indistinguishable. The *Brugia* microfilariae measure 111 to 230 μm in length (Plate 3.21). In stained blood smears, they can

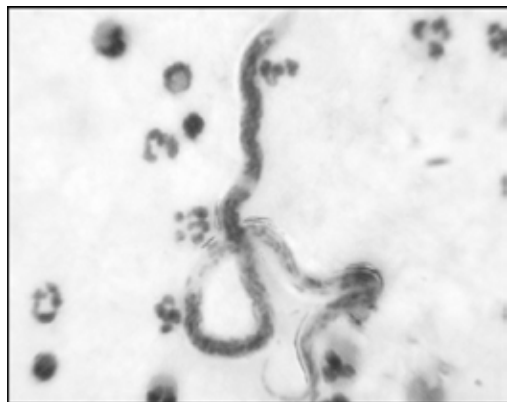


Plate 3.21. *Brugia malayi* microfilaria
(Courtesy of the Department of Parasitology,
UP-CPH)

be seen enclosed in a sheath, and having angular curvatures with secondary kinks, and two nuclei at the tip of the tail. The column of indistinct and confluent nuclei is composed of two rows.

Adult male and female *W. bancrofti* worms are found tightly coiled in nodular dilated nests (lymphangiectasia) in lymph vessels and in sinuses of lymph glands. Adult females produce microfilariae, which gain entrance to the peripheral blood circulation where they are picked up by the appropriate mosquito vector during a blood meal (Plate 3.22). Mosquitoes belonging to the genera *Aedes*, *Culex*, and *Anopheles* have been shown to be biologic vectors of *Wuchereria*. Microfilariae ingested by the mosquito migrate to its muscles where they develop into first (L1), second (L2), and third (L3) stage larvae. After 6 to 20 days of development, 3rd stage larvae force their way out of the muscles, causing considerable

damage, and migrate towards the mosquito's head and proboscis. During a blood meal, larvae emerge from the proboscis onto the skin of the susceptible host and actively penetrate the skin through the bite wound to reach the lymphatic vessels and nodes where they develop into adult worms. They are usually localized in the lymph vessels of the lower extremities, inguinal lymph nodes, epididymis of males, and labia of females. Microfilariae migrate from the parent worm, through the walls of the lymphatics, and into the neighboring blood vessels.

The life cycle of *B. malayi* generally follows the same pattern as that of *W. bancrofti* with a few exceptions (Figure 3.10). Mosquito vectors of *B. malayi* belong to the genus *Mansonia*. Development of the microfilariae to the infective stage in the mosquito takes about 2 weeks. Maturation time for the 3rd stage larvae to become adults takes about 3 to 9 months. Thereafter, microfilariae are produced and may be seen in the circulation.

Pathogenesis and Clinical Manifestations

LF is characterized by a wide spectrum of clinical manifestations, with signs and symptoms different from one host to another. The infection is usually acquired in childhood but may take years to manifest itself. The clinical course may be divided into asymptomatic, acute, and chronic stages, generally progressing in that order. In an endemic community, the different stages of the disease frequently overlap, and in certain groups of people from non-endemic areas, the disease may be characterized by an initial acute stage followed directly by a chronic stage in a relatively short period of time.

Individuals who grew up outside regions endemic for these filarial parasites and who get infected by them after migration to the endemic regions may clinically present with "Expatriate Syndrome." The syndrome is characterized by clinical and immunologic hyper-responsiveness to the mature or maturing worms. Together with the usual acute manifestations of lymphadenitis



Plate 3.22. *Wuchereria bancrofti* microfilaria
(Courtesy of the Department of Parasitology,
UP-CPH)

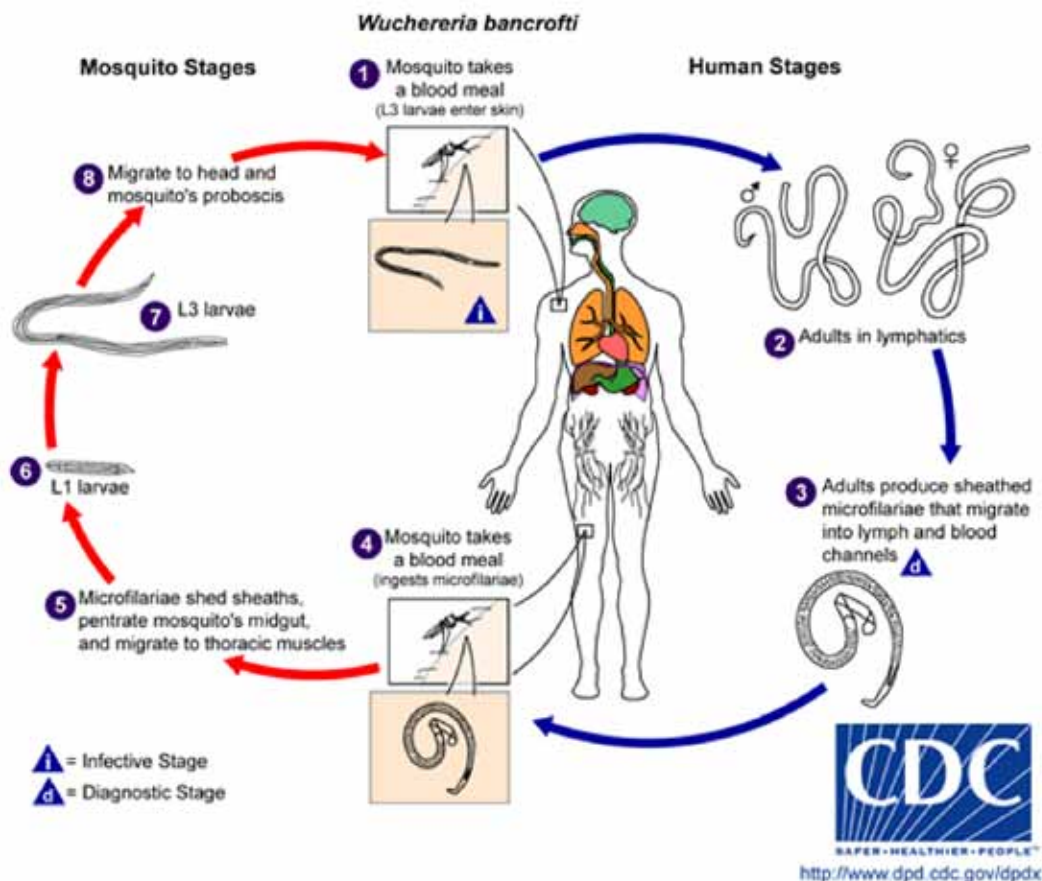


Figure 3.10. Life cycle of *Wuchereria bancrofti*
(Accessed from www.dpd.cdc.gov/dpdx)

and lymphangitis, individuals with this syndrome also present with allergic reactions such as hives, rashes, and blood eosinophilia.

Lymphatic localization is important in parasite survival because lymph is a less aggressive medium than blood: no platelets, no complement system, incomplete coagulation system, and no granulocytes; in addition, its flow is much less violent. Filarial adult worms cause parasite-induced lymphatic dilatation (lymphangiectasia); this is a common feature of patent infection, though clinically apparent lymphedema is rarely seen. Another cardinal feature of LF is lymphangiogenesis, where live filarial parasites or filarial antigens induce

lymphatic endothelial cell proliferation and differentiation leading to collateralization. These lymphatic dysfunctions have been shown to predispose infected individuals to secondary bacterial infections and trigger inflammatory reactions in the skin and subcutaneous tissue, leading to lymphedema and elephantiasis.

A characteristic feature of chronic LF infection is fibrosis and cellular hyperplasia in and around the lymphatic walls; these changes are postulated to render lymphatic endothelial cells less effective at transporting interstitial fluid, thereby contributing to the edema and collagen accumulation. Dead and decalcifying adult worms elicit immune

responses leading to lymphatic blockage and gross pathological lesions; it invokes lymphangitis and lymphadenitis with localized pain and swelling. The amount of exposure to secondary bacterial infections and the magnitude of host immunity to infective or developing larvae, or to *Wolbachia* increase the risk of development of chronic disease. Lymphatic insufficiency leads to increase susceptibility to opportunistic infections, and result in acute dermatolymphangioadenitis (ADLA) (Plate 3.23). Another potent inducer of inflammation is exposure to *Wolbachia* that is released by dead or dying worms.



Plate 3.23. Dermatolymphangioadenitis
(acute lymphatic filariasis)
(Courtesy of Dr. Vicente Belizario, Jr.)

The clinical spectrum of LF includes (a) asymptomatic microfilaremia, (b) acute dermatolymphangioadenitis (ADLA) also previously called adenolymphangitis (ADL), (c) acute filarial lymphangitis (AFL), (d) lymphedema and elephantiasis, (e) genito-urinary lesions (e.g., hydrocele), and (f) tropical pulmonary eosinophilia (TPE).

One of the most striking features of LF is that individuals with thousands to millions of vigorously motile microfilariae in the peripheral blood often show no obvious clinical signs of disease, known as asymptomatic microfilaremia. These individuals serve as the main reservoir for mosquito vectors which acquire microfilariae during a blood meal. This stage is characterized

by several immune regulatory processes driven by living parasites to ensure their long-term survival. Co-infection with other parasites and infectious disease is common, and the suppressive immunomodulatory mechanisms by the worm can modulate protective immune responses for malaria and tuberculosis. Though no clinical manifestations are seen and they appear outwardly healthy, these individuals may actually have hidden lymphatic pathology and kidney damage. Recent studies in animals show direct evidence that infection with *Brugia* can selectively induce CD4+ lymphocyte apoptosis, which may contribute to immune unresponsiveness to filariasis. The asymptomatic stage may also be seen in those individuals who are called “endemic normals,” who harbor in their blood the parasite antigen instead of the microfilariae.

ADLA is the most common acute manifestation of LF, defined as localized pain, lymphadenitis and/or lymphangitis and/or cellulitis and local warmth, with or without systemic manifestations of fever, nausea, and vomiting. Clinical descriptions are remarkably similar to those of erysipelas and cellulitis. The attacks are recurrent, and among patients in LF-endemic areas, the mean annual reported incidence ranges from 1.5 to more than 7 episodes per patient. The duration of symptoms, based on patient self reporting, ranges from 1 to 16 days, which result in significant short term disability, where the number of workdays lost may exceed the duration of the ADLA episode itself. Studies indicate that the rate of ADLA is higher in persons with chronic disease, particularly lymphedema. Among those with lymphedema, the risk factors for ADLA include increasing patient age, poor hygiene, and illiteracy. Studies from Brazil, India, and Guyana show that the presence and number of interdigital skin lesions are very strong risk factors for attacks of ADLA.

Current evidence shows that ADLA is of bacterial etiology, based on clinical signs

and symptoms (erysipelas or cellulitis-like), and isolation of bacteria at the time of the acute episode. The bacteria most frequently associated with ADLA episodes are Group A *Streptococcus*, although other bacteria are often found in cultures, including non-pathogenic strains. Thus, secondary bacterial infections from neglected skin lesions (reduced sensation predisposes to trauma, and poor hygiene) precipitate attacks of ADLA, and repeated ADLA episodes are deemed the most important factor in lymphedema progression.

AFL is a rare manifestation directly caused by adult worms that died spontaneously, or commonly observed following treatment with diethylcarbamazine (DEC), the latter is considered evidence of the drug's macrofilaricidal efficacy. AFL is characterized by lymphangitis that progresses distally along the lymphatic vessel, producing a palpable "cord." AFL may be accompanied by mild fever, headache, and malaise. Distal lymphedema may occur, but it is usually mild and reversible. The symptoms are self-limited or generally subside without treatment.

The most common chronic manifestation of LF is lymphedema, which on progression leads to elephantiasis (Plate 3.24). The lower limbs are commonly affected, but upper limb and male genitalia may be involved. In females, breasts and genitalia may be affected, but this is relatively uncommon. Repeated ADLA episodes are responsible for lymphedema progression and elephantiasis. Literature on lymphedema in filariasis-endemic areas lack standardization in terms of terminology, agreed-upon criteria for diagnosis, and case definition. Many authors use the term 'elephantiasis' for all forms of lymphedema.

Dreyer et al. in 2002 proposed a staging system for chronic lymphedema. In stage 1, the swelling increases during the day but is reversible once the patient lies flat in bed. In stage 2, the swelling is no longer reversible overnight, and the patient may still experience



Plate 3.24. Elephantiasis
(Courtesy of Dr. Vicente Belizario, Jr.)

acute attacks. The main feature of stage 3 is the presence of shallow skin folds, these are folds where the base can still be seen when the patient moves the leg or foot and the fold "opens up." Lines or creases not seen in the normal leg are already considered shallow folds. In stage 4, there are knobs present in the affected area; these are lumps or protrusions in the skin that predispose the area to trauma. A patient in stage 5, has deep skin folds, where the base can no longer be seen when the patient moves the leg, but only when the folds are actively "opened" by hand. In stage 6, mossy lesions are present, brought about by the clustering of small elongated or rounded growths. These usually leak translucent fluid, putting the area at risk for secondary bacterial infection. In stage 7, the patient is unable to adequately or independently perform activities of daily living due to the extent of the pathology. The infected area is foul-smelling and the affected individual frequently experiences acute attacks.

Hydrocele or chylocele results in the obstruction of the lymphatics of the tunica

vaginalis (Plates 3.25–3.26). Clear or straw-colored hydrocele fluid typically accumulates in the closed sac of the testis, and rarely, the fluid may have a milky appearance caused the presence of lymph—a condition known as chylocele. Hydrocele is a common chronic disease manifestation of Bancroftian filariasis since *W. bancrofti* worms have been shown ultrasonographically to prefer localization in scrotal lymphatics. These cases usually occur after puberty, and the prevalence increases with age. Chronic epididymitis, funiculitis, lymphedematous thickening of the scrotal skin, and thickening of the spermatic cord are also genital manifestations of chronic Bancroftian filariasis. The thickened cord can usually be palpated during physical examination. In females, lymphedema of the vulva may occur.



Plate 3.25. Hydrocele
(Courtesy of Dr. Vicente Belizario, Jr.)

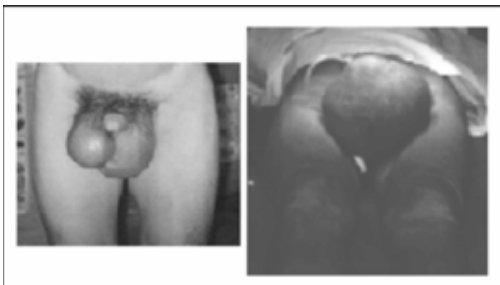


Plate 3.26. Small and big hydroceles in two patients suffering from filariasis
(Courtesy of Dr. Vicente Belizario, Jr.)

Although Malayan filariasis occasionally presents with groin involvement, hydroceles are rare. Deformities resulting from Malayan filariasis are not as severe as in Bancroftian filariasis. There may be enlargement of the epitrochlear, inguinal, and axillary lymph nodes. More advanced cases may either be asymptomatic, or may manifest with elephantiasis of one or more limbs, usually involving the area below the knee or below the elbow.

Rupture of lymphatics in the kidney may produce chyluria. This results from the blockage of retroperitoneal lymph nodes below the cisterna chyli. There is consequent reflux and flow of the intestinal lymph directly into the renal lymphatics, which may rupture and allow the flow of chyle into the urinary tract. The “milky urine” contains considerable quantities of lymph originating from the gastrointestinal tract. There are several reports of glomerulonephritis in patients with Bancroftian filariasis. Microscopic hematuria may also occur in microfilaremic persons.

Tropical pulmonary eosinophilia (TPE) is a classic example of occult filariasis in which the typical clinical manifestations are not present, and microfilaria are not found in the blood but may be found in the tissues. The syndrome, which is brought about by immunologic hyper-responsiveness to filarial infection, is characterized by paroxysmal nocturnal cough, hypereosinophilia (3,000–5,000 cells per mm³ of blood, levels unrelated to the severity of symptoms), elevated erythrocyte sedimentation rate, evidence of diffuse miliary lesions or increased bronchovascular markings, extremely high titers of filarial antibody (IgE), and good therapeutic response to DEC. In most cases, lung function is impaired, with a reduction in vital capacity, total lung capacity, and residual volume. It is commonly misdiagnosed as asthma or tuberculosis. Chronic symptoms may delay diagnosis, and if untreated, TPE progresses to chronic pulmonary fibrosis and respiratory failure.

Diagnosis

The microscopic finding of characteristic microfilaria in the blood is the traditionally accepted procedure. Due to the nocturnal periodicity of most *W. bancrofti* strains, wet smears or thick blood smears are taken between 8 p.m. and 4 a.m. In many chronic infections, microfilariae may not be demonstrable in the peripheral blood. This may be brought about by the following factors: (a) low intensity of infection, (b) dead worms, and (c) obstructed lymphatics. In cases of low intensity infections, filtration using a nucleopore filter or the Knott's method for concentration may be used. Table 3.3 summarizes the main distinguishing features of the microfilariae of *W. bancrofti* and *B. malayi* which may be appreciated microscopically in stained thick blood films. The DEC provocative test (3 mg /kg DEC single dose) stimulates

microfilariae into coming out to the peripheral circulation, allowing blood smear collection even during daytime.

Although these methods are still widely used, their low sensitivity and poor acceptability necessitate alternative approaches that fulfill the requirements for control program mapping, monitoring and assessment, and endpoint decision criteria and surveillance. Detection of circulating filarial antigens (CFA) is now the preferred method since it also detects latent infections. This is mainly done with immunochromatographic card tests. These simple card tests that detect CFAs are very sensitive and specific, thus eliminating the need for laboratory facilities. Other diagnostic approaches include molecular xenomonitoring of parasites in pools of mosquitoes, and detection of exposure to transmission in children with antibody detection.

Table 3.3. Comparison of microfilaria of *Wuchereria bancrofti* and *Brugia malayi*

	<i>Wuchereria bancrofti</i>	<i>Brugia malayi</i>
Mean length (μm)	290	222
Cephalic space : breadth	1:1	2:1
Sheath in Giemsa	Unstained	Pink
Nuclei	Regularly spaced, separately situated	Irregularly spaced, and overlapping
Tail	Single row of nuclei that does not reach the tail's end	Single row of nuclei that reaches the tail's end
Terminal nuclei	None	2 nuclei, which bulge the cuticle, conspicuously placed
Appearance in blood film	Smoothly curved	Kinky
Innenkörper length (μm)	34	30.7

Source: World Health Organization. Control of lymphatic filariasis: a manual for health personnel. Geneva: World Health Organization; 1987.

Treatment

DEC has been the drug of choice for the treatment of lymphatic filariasis since its discovery in 1948. It is effective against both microfilaria and adult worms; however, some strains of adult worms may not be sensitive to the drug. It markedly lowers blood microfilaria even in single once-a-year doses of 6 mg/kg. This reduction is sustained for about one year,

and is the basis of preventive chemotherapy for the interruption of transmission in elimination programs. A single optimum dose of DEC does not clear all microfilariae and does not kill all adult worms. A regimen of 6 mg/kg for 12 consecutive days is better than the single dose, and can be given to individuals if supervised by a medical practitioner, preferably in divided doses after meals.

The drug's mechanism of action is not well understood, but it is clear that host components are necessary, such as the arachidonic acid pathway and the 5-lipoxygenase pathway. Recent trials show that DEC has no role in the treatment and prevention of ADLA attacks in lymphedema. DEC is the treatment of choice for the treatment of TPE and is given for 3 to 4 weeks.

Adverse events (AEs) include fever, myalgia, headache, and sore throat or cough lasting 24 to 48 hours. These are mild and self-limiting, and may be treated symptomatically. These AEs represent an immune response that is mainly due to the destruction of microfilaria that is similar to the Mazzotti reaction seen in onchocerciasis. There may also be AEs associated with rapid killing of adult worms (AFL), which can lead to scrotal pain in men, and systemic inflammation due to the release of *Wolbachia*. Direct adverse events due to the drug are rare.

Ivermectin is a drug primarily used in the treatment of onchocerciasis, loiasis, and strongyloidiasis. It is also effective against ectoparasites such as lice and scabies. Used in LF, it is highly effective and well tolerated at doses of 100 to 200 µg/kg for the reduction of microfilaremia for up to 1 year. Ivermectin leads to hyperpolarization of glutamate-sensitive channels and immobilization of microfilaria. AEs are similar to DEC but milder due to its relatively slower parasite clearance. It has no proven action against adult worms and TPE.

Albendazole is a broad-spectrum anthelmintic given orally that is effective against nematodes, cestodes, and flatworms. Its mechanism of action is via inhibition of polymerization of β -tubulin and microtubule formation. A low dose of 400 mg used for the treatment of most intestinal helminth infections decreases *W. bancrofti* microfilaremia progressively for 6 to 12 months. Based on current studies, combination with DEC or ivermectin reduces microfilarial loads in the periphery longer than treatment with DEC or

ivermectin alone. There is probably no added effect against adult worms in LF. DEC or ivermectin in combination with albendazole used in LF elimination programs has the added benefit of clearing soil-transmitted helminth (STH) infections.

Doxycycline and related antibiotics kill the endosymbiont *Wolbachia*, which is essential for growth, development, embryogenesis, and survival of filarial worms. Treatment of LF with a course of doxycycline at 200 mg daily for 4 to 6 weeks results in long-term sterility and eventual death of adult worms. Anti-*Wolbachia* therapy showed significant improvements in lymphatic pathology and a decrease in the severity of lymphedema and hydroceles. Studies have also shown that prior treatment with doxycycline reduces the frequency and severity of AEs to DEC-albendazole. This relatively good safety profile is due to the avoidance of parasite-mediated or *Wolbachia*-mediated inflammatory adverse reactions. Although anti-*Wolbachia* chemotherapy has many benefits especially in the treatment of individual patients, its use in community-based control and elimination programs is hindered by the logistics of the length of treatment and contraindications in children and pregnant women.

The treatment recommendations for ADLA include bed rest, cooling the affected area to relieve the pain, analgesics and antipyretics for pain and fever, topical antibiotics and antifungals for superficial bacterial and fungal infections, systemic antibiotics (e.g., penicillin) for moderate to severe cases, and elevation of the involved extremity. Enrollment in a hygiene education program dramatically reduces the incidence of ADLA and the progression of lymphedema to elephantiasis. A proper "foot care program" includes: (a) washing the affected limb twice a day with soap and water especially the webs of toes and skin folds, and drying with a clean cloth to remove moisture; (b) clipping nails often and keeping them clean; (c) preventing and promptly treating local injuries and infections with topical agents; (d) regular

use of properly fitting footwear; and (e) raising the affected limb at night to reduce the swelling.

In the setting of severe lymphedema and elephantiasis, the hygiene education program stated above may be supplemented with the use of compressive bandages, stockings, manual lymphatic drainage (massage), heat therapy, and, in refractory cases, surgical procedures.

An estimated 27 million males suffer from hydroceles, and the prevalence is strongly associated with the intensity of parasite transmission (microfilaremia prevalence). Recent observations from Brazil, Egypt, and Haiti indicate that many acute hydroceles resolve spontaneously, and about 24% persist to become chronic. Surgery is the recommended treatment for hydrocele, and if done properly, is deemed curative. Other methods such as aspiration of fluid and injection of sclerosing substances are less effective, are associated with hydrocele recurrence, have unacceptable side

effects, and have not been adequately evaluated in filariasis-endemic areas. Current WHO guidelines call for the complete surgical removal of the tunica vaginalis to minimize or prevent recurrence.

Epidemiology

About 120 million people worldwide are affected by the disease, and more than 1 billion people are at risk (one-fifth of the world's population), mostly in the poorest areas. Bancroftian filariasis accounts for 90% of cases in 83 endemic countries while the Malayan filarial worm (and *B. timori*) causes the remainder. *W. bancrofti* affects more than 100 million people in the tropical areas of India, Southeast Asia, the Pacific Islands, Africa, and South and Central America. India has the largest number of cases. *B. malayi* and *B. timori* affect 12.5 million people in Southeast Asia (Figure 3.11). The prevalence of infection continues to

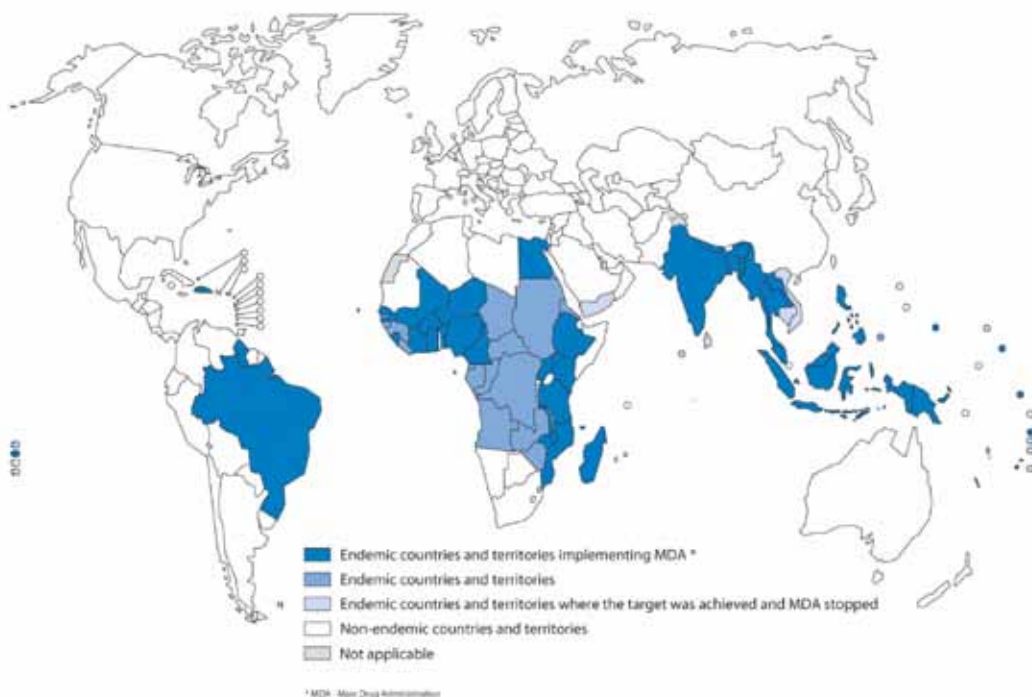


Figure 3.11. Distribution and status of preventive chemotherapy for lymphatic filariasis, worldwide, 2010 (Accessed from gamapserver.who.int)

rise in tropical and subtropical countries due to rapid growth of cities. This creates more breeding sites for mosquitoes to transmit the disease in areas where *Culex* is the vector.

In rural areas, particularly in Africa, *W. bancrofti* is transmitted by the *Anopheles* mosquito, which includes species that transmit malaria. In urban areas, the major vectors are *Culex* mosquitoes which can breed in latrines, sewage, and ditches. In the Pacific region, mosquito vectors belonging to the genus *Aedes* can breed in tiny areas of clean water in the axils of plants (Plates 3.27–3.28), empty containers, or old tires.

In the Philippines, 45 provinces are endemic for LF: (Region IV) Quezon Province, Marinduque, Oriental Mindoro, Occidental Mindoro, Palawan, and Romblon; (Region V) Albay, Camarines Norte, Camarines Sur, Catanduanes, Masbate, and Sorsogon; (Region VI) Aklan, Antique, Capiz, and Iloilo; (Region VII) Negros Oriental; (Region VIII) Biliran, Eastern Samar, Northern Samar, Northern



Plate 3.27. Farmer in abaca plantation
(Courtesy of Dr. Vicente Belizario, Jr.)

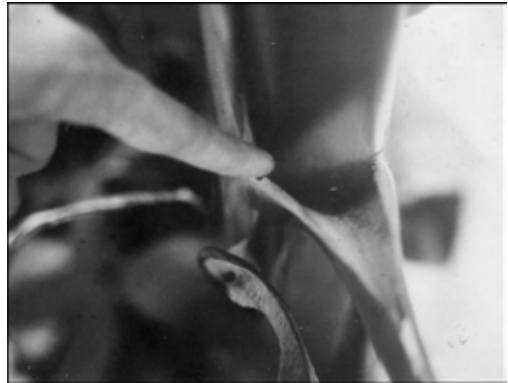


Plate 3.28. An axil of abaca:
a breeding site of *Aedes poecilus*
(Courtesy of Dr. Vicente Belizario, Jr.)

Leyte, Southern Leyte, and Western Samar; (Region IX) Zamboanga del Norte, Zamboanga Sibugay, and Zamboanga del Sur; (Region X) Bukidnon, Misamis Occidental, and Misamis Oriental; (Region XI) Compostela Valley, Davao del Norte, Davao del Sur, and Davao Oriental; (Region XII) North Cotabato, Sarangani, South Cotabato, and Sultan Kudarat; (CARAGA) Agusan del Norte, Agusan del Sur, Dinagat Islands, Surigao del Norte, and Surigao del Sur; (ARMM) Basilan, Maguindanao, and Sulu (Figure 3.12).

Aedes poecilus, which breeds in water accumulated in the axils of abaca and banana plants, is the mosquito vector in most provinces of the Philippines. *Anopheles minimus* var. *flavivirostris*, the principal vector for malaria in the Philippines is also the vector of *W. bancrofti* in Sulu and Palawan. Malayan filariasis has been described in Palawan, Eastern Samar, Agusan del Sur, and Sulu. In these places, *W. bancrofti*, co-exists with *B. malayi*. The mosquito vectors are *Mansonia bonnae* which breeds in freshwater swamps, and *Mansonia uniformis* which breeds in rice fields. These mosquitoes are night biters and they usually start biting as early as 5 p.m. until 11 p.m. The reported prevalence is less than 3%. Cats are important reservoir hosts and may transmit the infection to humans by means of the cat-mosquito-man cycle.

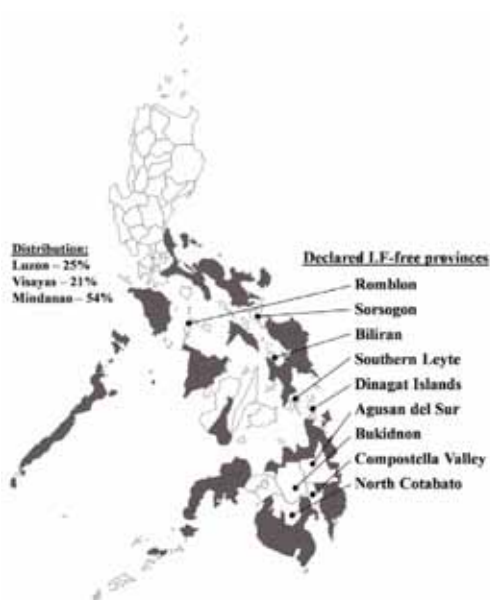


Figure 3.12. Map of lymphatic filariasis-endemic provinces in the Philippines, distribution in the three major island groups, and provinces declared lymphatic filariasis-free by the Department of Health
(Adapted from www.doh.gov.ph/content/national-filariasis-elimination-program)

The national microfilaria rate (MFR) in 1998 was 9.7%. Although the reported prevalence rates appear to be generally low—below 3%, studies in Sorsogon have shown that microfilaria rates may be as high as 15% in endemic villages. In a village in Sorsogon, hydrocele was present in 4% of males, while incidence of ADLA over a 1-year follow-up period was 100 cases per 1,000 population. Recent studies show that Romblon province has the highest CFA rate of 18.8%, and Oriental Mindoro has the highest microfilaria prevalence rate of 12.6%.

In the Philippines, areas endemic for LF are in regions with the highest incidence of poverty. Out of a total of 80 provinces, 39 have a higher poverty incidence than the national average and 30 of these 39 provinces are endemic for LF.

In general, adults are more frequently infected than children, and there are more

infected males than females. This may be due to economic activities (e.g., abaca farming) that increase exposure of adult males to mosquito vectors. In the Bicol region, hydroceles are more frequently encountered than elephantiasis of the extremities.

Prevention and Control

The World Health Organization (WHO) in the 50th World Health Assembly has targeted LF for elimination by the year 2020. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has two major goals: to interrupt transmission of the parasite via preventive chemotherapy, and to provide care for those who suffer from the clinical manifestations of LF through hygiene education programs. The development of safe, effective, and well-tolerated single dose microfilaricidal regimens has resulted in effective and sustainable drug delivery in endemic areas. DEC-medicated table or cooking salt has been used successfully in eliminating LF in some endemic areas. Besides the commonly used filaricidal drugs, drug development is continuously being undertaken. Moxidectin has been proven in recent animal trials to be a very effective macrofilaricide.

The goal for endemic communities is to eliminate the presence of microfilariae in the blood in order to prevent transmission of the disease by vectors. According to the WHO, single doses of DEC in combination with another drug such as albendazole or ivermectin is 99% effective in removing microfilariae from the blood for up to one year from treatment. Proper control of transmission in communities therefore entails the identification of endemic areas and implementation of mass treatment programs using an albendazole/DEC combination; or a DEC/ivermectin combination in areas where onchocerciasis or loiasis is prevalent. The use of albendazole/DEC or albendazole/ivermectin combinations offers opportunities for integrated control of STH and LF.

In the Philippines, the four provinces in Panay Island as well as the province of Quezon were recently found to be endemic. The Department of Health (DOH) is currently implementing MDA activities in those provinces. According to the DOH, nine provinces have reached elimination level: Southern Leyte, Sorsogon, Biliran, Compostela Valley, Bukidnon, Romblon, Agusan del Sur, Dinagat Islands, and North Cotabato. The criteria for a province to be declared LF-free are: (a) MFR of <1%; (b) no true positives in children ages 2 to 4 years old; and (3) no true positives among new school entrants. MDA coverage rates for the monitoring and evaluation of elimination programs should be used with caution. A study by Amarillo, et al. in 2008 revealed over-reporting, where the proportion of the sampled population that received and ingested the antifilarial drugs was much lower than the reported coverage.

Personal protective measures may help prevent contact with mosquito vectors. The use of mosquito nets as well as insecticide residual spraying may help decrease the number of mosquito vectors at home. In addition, advances in vector control include the development of *Bacillus sphaericus* sprays and polystyrene beads to seal latrines in order to eliminate or reduce *Culex* vector populations. Health education may also benefit those who, living in endemic areas which may lack awareness on the etiology, prevention, and control of LF.

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Parastrongylus cantonensis

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes

Previously classified under the genus *Angiostrongylus*, *Parastrongylus cantonensis*, or the rat lungworm, was first described by Chen in 1935 from domestic rats in Canton, China. The nematode, which normally lives in rat lungs, has been known to cause eosinophilic meningoencephalitis in man. Human infection was first reported in Taiwan in 1945. Parastrongyliasis outbreaks in the Pacific islands have been documented since then, and more than 2,800 cases have already been reported worldwide.

Parasite Biology

The adult worm, which is pale and filiform, has a length of 17 to 25 mm (Plate 3.29). Male worms measure 16 to 22 mm in length and 0.25 to 0.35 mm in diameter. They have a well-developed caudal bursa, which is kidney-shaped and single-lobed. Female worms measure 19 to 33 mm in length and 0.28 to 0.50 mm in diameter. The female worms have uterine tubules that are wound spirally around the

intestine. This arrangement is usually described as the “barber’s pole” pattern. The morphologic features may be observed through the worm’s transparent cuticle. The posterior end of the female worm is blunt shaped. A single female worm can lay up to 15,000 eggs daily.

The elongated ovoidal eggs have delicate hyaline shells. They measure 46 to 48 μm by 68 to 74 μm and are unembryonated when oviposited. The 1st stage larva, found in the lungs of the rodent host, has a distinct small knob near the tip of the tail. Two well-developed chitinous rods below its buccal cavity identify the third stage larva. These rods have expanded knob-like tips.

Rats are the definitive hosts of *P. cantonensis*. Rats are infected through ingestion of the third stage larvae. The larvae penetrate the stomach wall and travel in the bloodstream until they reach the central nervous system. They undergo two molts, which take about 2 weeks, before they reach maturity. Early development occurs in the brain. After the final molt in rats, the young adults migrate to the pulmonary arteries to complete their development. After 2 weeks, the adult females start laying eggs.

Adult worms live in the two main branches of the pulmonary arteries of the rat. In the bloodstream, gravid females lay eggs, which are transported into the smaller vessels of the lungs. After 6 days, eggs hatch and release the first stage larvae that penetrate into the respiratory tract. The larvae then migrate up to the trachea and reach the oropharynx where they are then swallowed and eventually expelled in the feces. It takes about 6 to 8 weeks from infection before the rat excretes 1st stage larvae (Figure 3.13).

The first stage larva is the infective stage for the molluscan intermediate host. In the Philippines, the known intermediate hosts

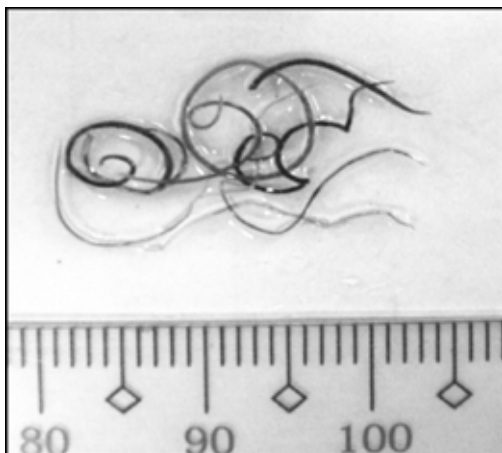


Plate 3.29. *Parastrongylus* adults
(Courtesy of the Department of Parasitology,
UP-CPH)

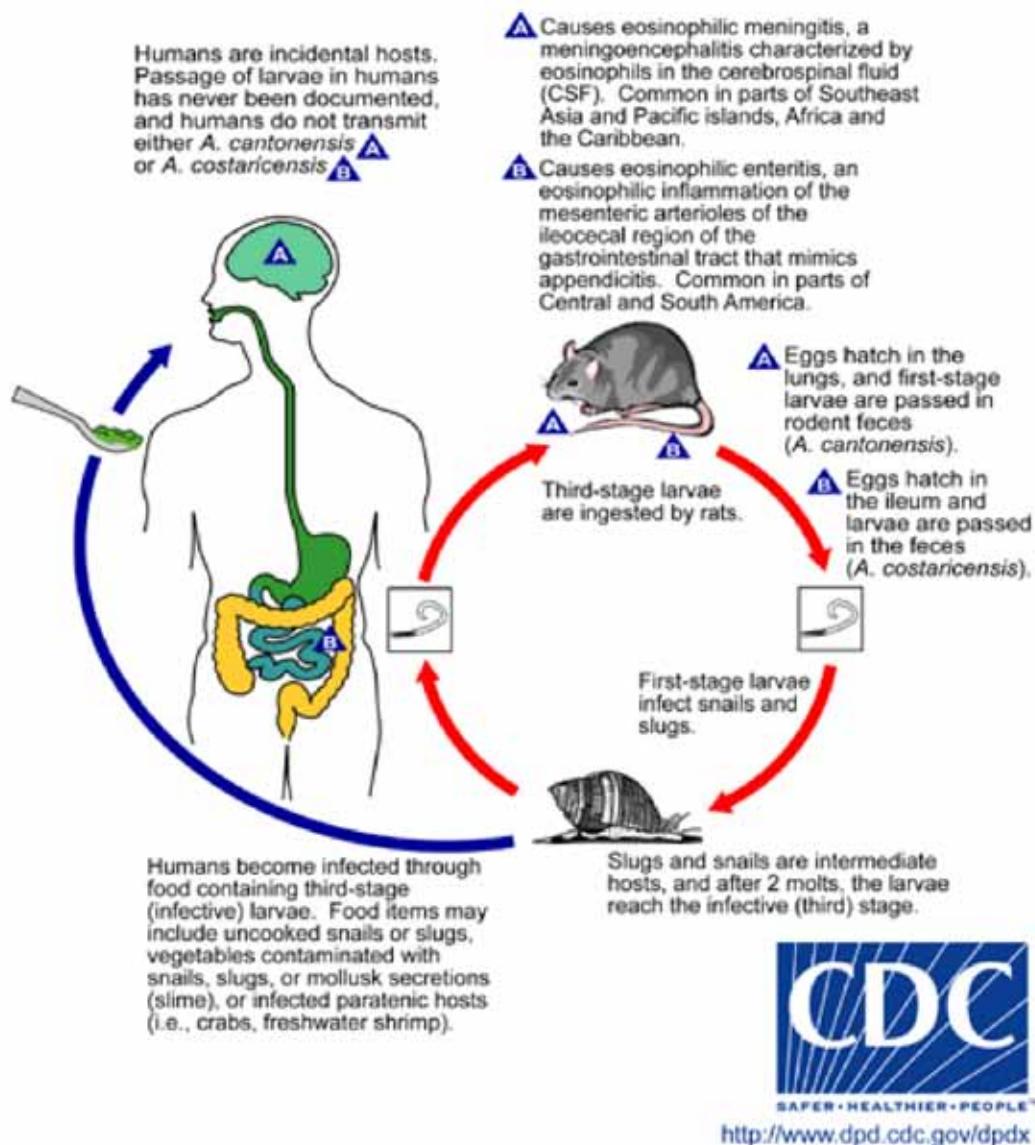


Figure 3.13. Life cycle of *Parastrongylus cantonensis*
 (Accessed from www.dpd.cdc.gov/dpdx)

include the following slugs and snails: *Achatina fulica* (Plate 3.30) or giant African snail, *Hemiplecta sagittifera*, *Helicostyla macrostoma*, *Vaginilus plebeius*, and *Veronicella altae*. Its mode of infection is by ingestion or active penetration. In the mollusk, larva eventually develops into the 3rd larval stage in about 12 days.

Although the mechanism by which humans get infected is not yet entirely clear, transmission is usually attributed to: (a) ingestion of the raw mollusk intermediate host infected with the third stage larva; (b) ingestion of leafy vegetables contaminated with mucus secretions of the mollusk carrying the infective stage (3rd larval



Plate 3.30. *Achatina fulica*, the intermediate host of *Parastrongylus cantonensis*
(Courtesy of the Department of Parasitology, UP-CPH)

stage) of the parasite; (c) ingestion of a paratenic host, such as freshwater prawn or crab harboring the infective stage of the parasite; or (d) drinking of contaminated water.

When humans get infected, the larvae pass through the stomach into the intestine, enter the circulatory system and migrate to the brain or spinal cord, or occasionally migrate into the eye chamber. In humans, however, the larvae probably remain in the brain for a longer period of time and do not develop to the adult stage.

Pathogenesis and Clinical Manifestations

In most cases, the incubation period is around 6 to 15 days, but may vary from 12 to 47 days. The chief complaint in many cases is acute, severe, intermittent occipital or bitemporal headache. Other common symptoms include stiffness of the neck, paresthesia, vomiting, fever, nausea, blurred vision or diplopia, body or muscle pain, and fatigue. Confusion, incoherence, disorientation, memory lapses, or coma have also been observed during illness. Intraocular hemorrhage and retinal detachment as associated complications have also been reported. Postmortem examination may show leptomeningitis, encephalomalacia and moderate ventricular dilation. Immature

worms may also be seen in the cerebrum and cerebellum. Eosinophils, monocytes, and foreign body giant cells in the spinal cord or in the cerebrospinal fluid (CSF) are usually associated with the infection. The CSF usually contains 100 to 1,000 leukocytes per μL . Adult worms have also been recovered from the eyes and pulmonary arteries of patients. Large numbers of Charcot-Leyden crystals have also been demonstrated in the meninges. Dead worms can also result in inflammatory reaction and local tissue necrosis.

Prognosis is usually good. In most cases, the disease is mild and no hospitalization is necessary. The infection is self-limited and symptoms gradually disappear with recovery. Meningeal symptoms are often the first to subside, followed by improvements in vision, and relief from paresthesia. Cranial nerve involvement is the last to recover. Permanent neurologic deficits have been documented, and in rare cases, the disease may result in death.

Diagnosis

Diagnosis of parastrongyliasis in humans is relatively difficult, since the primary site of infection is the brain. Presumptive diagnosis may be made based on travel and exposure history, correlated with clinical symptoms, medical history, laboratory findings, brain imaging results, and serological tests.

Examination of blood may reveal a high proportion of eosinophils, comprising 7 to 36% of the white blood cell (WBC) count. Examination of CSF may contribute to increased sensitivity in the diagnosis of parastrongyliasis. CSF eosinophilia of greater than 10% in proportion to WBC will exclude other common causes of meningitis. The CSF protein level in most patients is mildly elevated, while the CSF glucose is normal. However, other infections (e.g., cysticercosis, trichinosis, visceral larva migrans, schistosomiasis, paragonimiasis, and gnathostomiasis) involving the central nervous system must first be ruled out.

Meningeal lesions may be appreciated with the use of computed tomography (CT) scan. CT scans may also reveal non-specific cerebral edema and ventricular dilatation. Magnetic resonance imaging (MRI) may show lesions with hyperintense T2 signal. Although enzyme-linked immunosorbent assay (ELISA) for the diagnosis of parastrongyliasis is still not commercially available, a dot-blot ELISA that tests blood has been demonstrated to be 100% sensitive and specific for use in epidemiological surveys. In addition, serum antigens from *P. cantonensis* can also be detected by immunopolymerase chain reaction (PCR).

Treatment

No anthelmintic treatment is recommended at present, although mebendazole and albendazole have been demonstrated to effectively treat parastrongyliasis in China, Taiwan, and Thailand. Anthelmintic therapy has been shown to relieve symptoms and reduce the duration of the disease. Ocular parastrongyliasis may require surgical removal of worms from the eyes. Symptomatic treatment with the use of analgesics and lumbar puncture can relieve the headaches experienced by the patient with eosinophilic meningitis. Prednisone 30 mg daily is recommended, particularly in severe cases with cranial nerve involvement. The anti-inflammatory and immunosuppressive effects of steroids are helpful in mitigating the disease process.

Epidemiology

Human infection with *P. cantonensis* was first reported in 1945 by Nomura and Lin in Taiwan. As a human parasite, *P. cantonensis* has also been documented in approximately 30 countries including Thailand, China, Tahiti, French Polynesia, USA, Cuba, New Caledonia, Japan, Australia, Vanuatu, India, and the Philippines.

In the Philippines, Nishimura and Yogore reported the presence of *Parastrongylus* in rats.

Further studies showed that its prevalence in rats is less than 7%. The presence of *P. cantonensis* as a parasite of rats and/or snails has been reported in the following provinces of Luzon: Batangas, Bulacan, Cavite, Ilocos Norte, Laguna, Mountain Province, Nueva Ecija, Pampanga, Pangasinan, Quezon, Rizal, Sorsogon, Tarlac, and Metro Manila. Two cases of ocular parastrongyliasis have been reported from the East Avenue Medical Center. The patients were blood relatives coming from Isabela who have eating history of improperly cooked snails. The worms were identified at the College of Public Health, University of the Philippines Manila.

Prevention and Control

The main preventive strategy against parastrongyliasis is through awareness and education on proper eating habits and safe food preparation. The public should be discouraged from eating raw or poorly cooked mollusks or unwashed vegetables. Hand washing after gardening should also be advised. Farmers occasionally use molluscicides, such as metaldehyde or iron phosphate food bait pellets to control intermediate hosts. Copper barriers against snails and slugs are also utilized by farmers to prevent contamination of vegetable and fruit crops. Health workers in endemic areas should also be educated on the diagnosis, treatment, control, and prevention of parastrongyliasis.

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Trichinella spiralis

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes

Trichinella was first described by Tiedemann in 1822. In 1835, James Paget and Richard Owen demonstrated *Trichinella* in human cadavers in London. Before the turn of the century, German investigators were able to prove that raw or insufficiently cooked meat (i.e., pork) was responsible for trichinellosis in humans. Trichinellosis was initially attributed to a single species, *T. spiralis*, but the discovery of marked strain differences in *Trichinella* isolates have led to the identification of new species.

There are eight recognized species and three genotypes under the genus *Trichinella*. *Trichinella spiralis* is the most important cause of trichinellosis in humans, and is the species that is most adapted to domestic and wild pigs. *Trichinella britovi*, on the other hand, is the most widely distributed species among wild animals in Asia, Europe, Northern Africa, and Western Africa, although it can also infect domestic pigs. *T. britovi* is the 2nd most common *Trichinella* species affecting humans. *Trichinella nativa* infects primarily wild carnivores in the frigid zones of Asia, North America, and North Eastern Europe. Other species that have been known to cause human trichinellosis include *T. murrelli*, *T. nelsoni*, *T. papuae*, and *T. pseudospiralis*.

Parasite Biology

The adult male, which measures 0.62 to 1.58 mm by 0.025 to 0.033 mm, has a single testis located near the posterior end of the body, and is joined in the mid-body by the genital tube which, in turn, extends back to the cloaca. The posteriorly-located cloaca has a pair of caudal appendages and two pairs of papillae. The adult female measures about 1.26 to 3.35 mm by 0.029 to 0.038 mm, and has a single ovary which is situated in the posterior part of

the body. In addition, the female worm has an oviduct, a seminal receptacle, a coiled uterus, a vagina, and a vulva. The vulva is situated in the anterior 5th on the ventral side of the body. The viviparous female lives for 30 days and is capable of producing more than 1,500 larvae in its lifetime.

The larva measures 80 to 120 μm by 5.6 μm at birth, but reaches the size of 0.65 to 1.45 mm in length and 0.026 to 0.040 mm in width after it enters a muscle fiber. It has a spear-like, burrowing anterior tip. The digestive tract of a mature larva encysted in a muscle fiber resembles that of the adult worm. The reproductive organs, at this stage, are not yet fully developed but even then, it is already possible to identify the sex of the parasite.

In *Trichinella* infection, the host (i.e., humans, rats, dogs, cats, pigs, bears, foxes, walruses, or any other carnivore or omnivore) serves as both the final and intermediate host by harboring both the adult and the larval stages. Infective larvae are usually encysted in the muscle fibers of the host (Plate 3.31).

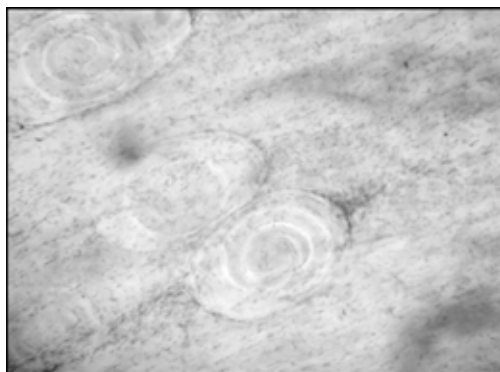


Plate 3.31. *Trichinella spiralis* larvae in muscle
(Courtesy of the Department of Parasitology,
UP-CPH)

The infective encysted larvae enter the host through ingestion of raw or insufficiently cooked meat. The cysts are digested in the stomach, and the larvae excyst either in the stomach or in the small intestine. The larvae then burrow into the subepithelium of the villi where they undergo four molts. Maturation takes about 2 days, and adult worms begin to mate 5 to 7 days post infection. The female produces eggs that grow into larvae in its uterus.

After a few days, the female worm deposits larvae in the mucosa. The larvae penetrate the mucosa, pass through the lymphatic system into the circulation, and finally into striated muscles (Figure 3.14). In the muscles, the larvae grow and develop. After about 3 weeks, they start to coil into individual cysts. Encapsulation is completed 4 to 5 weeks after infection. The larva in the cyst remains viable for many years. The average lifespan of the encysted larva is about 5

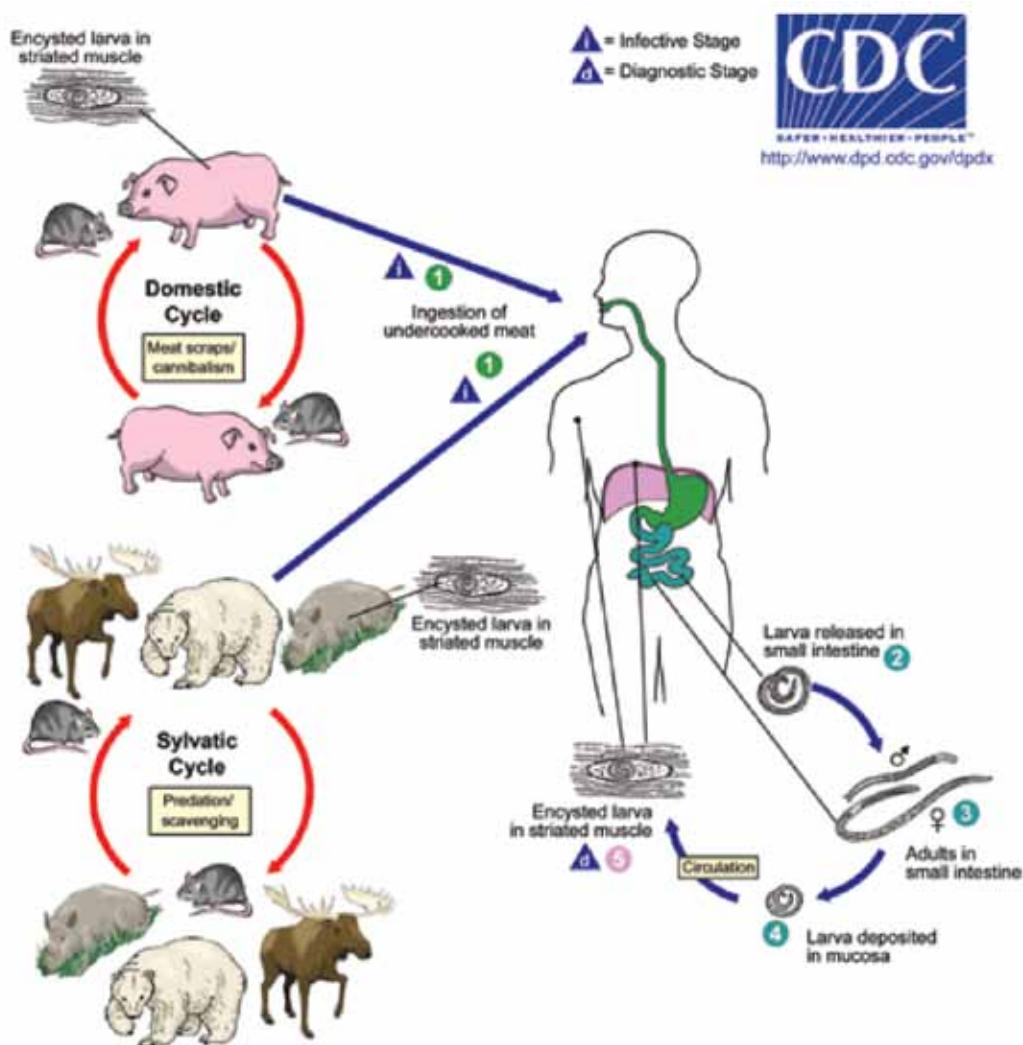


Figure 3.14. Life cycle of *Trichinella spiralis*
(Accessed from www.dpd.cdc.gov/dpdx)

to 10 years, and can survive for up to 40 years in humans. In humans, calcification of the collagen capsule in the infected muscle cell and the larva may occur. This process may be observed 6 to 12 months after infection and may lead to the destruction or death of the larva.

Pathogenesis and Clinical Manifestations

The severity of symptoms depends on the intensity of infection. Patients with light infection, i.e., harboring up to 10 larvae, are usually asymptomatic, while patients with moderate infection (50-500 larvae) show symptoms. Infection with a few hundred larvae can result in gastroenteritis, diarrhea, and abdominal pain approximately two days post infection. Infection with 100 to 300 larvae may lead to symptomatic trichinellosis, while more than 1,000 to 3,000 larvae can result in severe disease.

Clinical manifestations vary depending on the stage of the parasite. The clinical conditions are divided into three phases, namely: enteric phase, invasion phase, and convalescent phase. These correspond to the stages of: (a) incubation and intestinal invasion, (b) larval migration and muscle invasion, and (c) encystment and encapsulation.

Symptoms in the enteric phase may resemble those of an attack of acute food poisoning, including diarrhea or constipation, vomiting, abdominal cramps, malaise, and nausea. During the invasion phase, the migrating larvae and resulting metabolites lead to immunological, pathological, and metabolic reactions. Inflammatory reaction to the infection results in eosinophilia, which results in the release of histamines. Histamines, serotonin, bradykinins, and prostaglandins contribute to an increase in vascular permeability, resulting in tissue edema. The cardinal signs and symptoms of trichinellosis include severe myalgia, periorbital edema, and eosinophilia. Other typical signs and symptoms include high remittent fever and chills, headache,

dyspnea, dysphagia, and difficulty in chewing. Occasionally, there is paralysis of the extremities and splenomegaly. In severe cases, there may be gastric and intestinal hemorrhages.

Larval migration into the heart muscle can result in pericardial pain, tachycardia, and electrocardiogram abnormalities. Pericardial effusion, congestive heart failure, and other chronic heart abnormalities have also been observed. Neurological complications, which are caused by small subacute cortical infarcts, may occur in chronic infections. Meningitis and meningoencephalitis may also develop. In heavy infections, ocular disturbances, diplegia, deafness, epileptiform attacks, and coma may occur. In the convalescent phase, fever, weakness, pain, and other symptoms start to abate. Full recovery is expected since trichinellosis is a self-limiting disease. However, protean neurologic signs arising from brain damage may persist.

Prognosis is good, especially in mild infections. Death is uncommon except in cases of heart failure, encephalitis, or other complications such as pneumonia or septicemia. Low-grade or absent peripheral blood eosinophilia is indicative of poor prognosis.

Diagnosis

The most definitive diagnostic examination is the demonstration of the larva through muscle biopsy. Muscle biopsy is done through histological examination of 0.2 to 0.5 g of muscle tissue. Digestion of muscle samples with pepsin and hydrochloric acid can also be done to determine the number of larvae per gram of muscle, or to isolate larvae for molecular characterization. The digestion technique, however, is limited to muscle larvae that are about 10 to 12 days old (about 2-3 weeks post infection) since younger larvae may be destroyed by the digestion fluid.

Non-specific laboratory tests to detect eosinophilia, muscle enzymes (creatin phosphokinase, lactate dehydrogenase, and

myokinese), and total IgE in serum may be useful in diagnosis. An algorithm for the diagnosis of individual cases is shown in Table 3.4.

Table 3.4. Algorithm for the diagnosis of the probability of acute trichinellosis in humans

Group	Symptom
A	Fever, eyelid and/or facial edema, myalgia
B	Diarrhea, neurological signs, cardiac signs, conjunctivitis, subungual hemorrhages, cutaneous rash
C	Eosinophilia (> 1,000 eosinophils/ml) and/or increased total IgE levels, increased levels of muscular enzymes
D	Positive serology (with a highly specific test), seroconversion, positive muscular biopsy

The diagnosis of trichinellosis is very unlikely in the occurrence of only one symptom from group A, B or C. Trichinellosis may be suspected in the presence of one symptom from group A or two from group B, and one from group C, while a diagnosis is probable when there are three group A and one group C symptoms. Diagnosis is highly probable in the presence of three group A and two group C symptoms. A diagnosis is confirmed in case of three group A, two group C, and one group D symptoms; or any of symptom from group A or B, and one from group C and one from group D.

Currently, enzyme-linked immunosorbent assay (ELISA) is recommended for the diagnosis of trichinellosis. Confirmation of ELISA-positive samples may be done through Western blot technique. Latex agglutination technique may be utilized for rapid (<1 hour) confirmation of trichinellosis.

Treatment

The treatment of choice for trichinellosis is mebendazole 5 mg/kg body weight daily, or albendazole 15 mg/kg body weight per day in two divided doses, for 10 to 15 days. For

children 2 years and older, albendazole should be given at 10 mg/kg body weight. A treatment cycle may be repeated five days after the initial cycle in case of severe infection. Thiabendazole is no longer used due to its associated adverse drug reactions.

Supportive treatment through analgesics and antipyretics is commonly used to control symptoms. Corticosteroids may be given with anthelmintics to control hypersensitivity reactions to the larvae, and may also be given to treat acute vasculitis and myositis.

Epidemiology

Trichinella infections in humans have already been documented in 55 countries worldwide. There are about 10,000 cases reported each year, 0.2% resulting in mortality. Human trichinellosis occurs wherever meat is a part of the diet. Outbreaks have been reported in Argentina, Bosnia-Herzegovina, China, France, Laos, Romania, Spain, Sweden, Thailand, Turkey, Ukraine, Uzbekistan, and Vietnam. *Trichinella* infection has never been documented in a small number of island countries, including the Philippines.

Trichinellosis is primarily a zoonosis. Humans get infected after ingestion of raw or insufficiently cooked meat from infected animals. The infection is usually maintained in a pig-to-pig or pig-to-rat-to-pig cycle.

Prevention and Control

Health education is an important component of prevention and control measures against this parasitic infection. It is recommended that meat be cooked at a minimum of 77°C (170°F). Freezing is another way to kill larvae. Storage at –15°C for 20 days or –30°C for six days is suggested. Smoking, salting, or drying meat is not effective. Other control measures include regular animal monitoring (meat inspection or detection of circulating antibodies), keeping pigs in rat-free pens, and proper disposal of suspected carcasses.

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Anisakis spp.

Winifreda U. de Leon

Anisakids are nematode parasites of whales, dolphins, porpoises, walruses, seals, sea lions, and other deep marine mammals. Like any nematode, anisakids have elongated vermiform bodies without segmentation. They have a complete digestive tract, and the sexes are separate. Although they are parasites of marine mammals, they can cause gastrointestinal infections and allergic reactions in humans with the consumption of raw and undercooked squid and fish containing the 3rd stage larvae of the parasite. Commonly involved infective species are *Anisakis simplex* and *Pseudoterranova decipiens*. Related species include *Contracaecum* sp. and *Hysterothylacium* sp.

Parasite Biology

The adult worms embedded in the gastric wall of the marine mammal host discharge unembryonated eggs into the sea. The 1st stage larvae that develop inside the eggs molt into the 2nd stage larvae that hatch out of the egg. The free swimming 2nd stage larvae are ingested by micro-crustaceans, where the 3rd stage larvae develop. Going up the predatory food chain, the third stage larvae are transported to various paratenic hosts, like squid and several species of fish. Usually, the 3rd stage larvae are more concentrated in fish viscera but may occasionally be found in the fish muscles (Figure 3.15).

The 3rd stage larvae of *Anisakis simplex* are milky white in color, measuring 19 to 36 mm in length, with a long stomach, and a blunt tail with mucron, and are referred to as Type I larvae. Other species of *Anisakis* have third stage larvae with shorter stomachs and blunt tails, and are called Type II larvae. The 3rd stage larvae of *Pseudoterranova* are yellowish brown in color measuring 25 to 50 mm in length. Following

ingestion by marine mammals, the 3rd stage larvae molt twice and develop into adult worms.

Pathogenesis and Clinical Manifestations

Humans may ingest the 3rd stage larvae from raw or improperly cooked infected fish. The 3rd stage larvae, however, do not develop into the adults in the human gut. Larval infection with anisakids is called anisakiasis or, more recently, anisakidosis. It may result in gastric and intestinal pathology. A second manifestation of morbidity brought about by the parasites is an allergic reaction to the chemicals secreted by the worms.

Ingested larvae invade the submucosa of the stomach or the intestines, resulting in hemorrhage and inflammation. The larvae may die and detach. However, if the penetration is deep, a tumor-like granuloma surrounded by inflammatory cells and eosinophils will develop. Gastric anisakidosis is usually less acute and less exudative than the intestinal form.

Gastric anisakidosis has an acute presentation, occurring within 1 to 12 hours after ingestion of infective larvae. Most patients complain of severe abdominal pain accompanied by nausea and vomiting. The acute symptoms may eventually subside, with vague but persistent abdominal pain and intermittent bouts of nausea and vomiting. Occasionally, the larvae may be regurgitated. Symptoms may be mistaken for peptic ulcer disease, cholecystitis, or even gastroenteritis.

When the larvae pass into the intestines, a severe eosinophilic granulomatous response may occur 1 to 2 weeks following infection. Intestinal anisakidosis usually mimics appendicitis, Crohn's disease, intestinal obstruction, or diverticulitis.

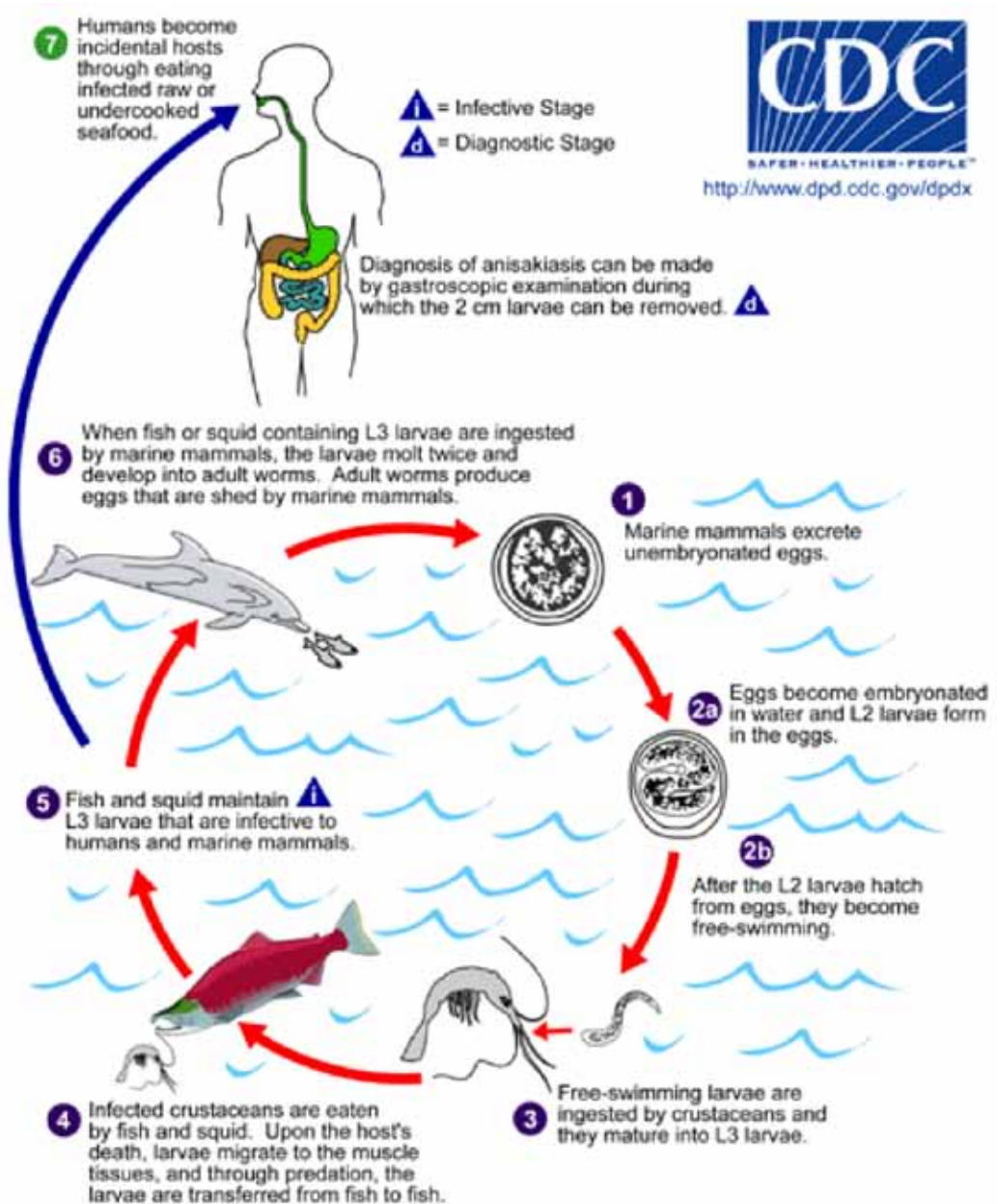


Figure 3.15. Life cycle of Anisakids
(Accessed from www.dpd.cdc.gov/dpdx)

Outside of these more common locations, the larvae have been found invading the oropharynx, esophagus, and colon. This condition is referred to as ectopic anisakidosis.

When the oropharynx is involved, the presentation is commonly known as “tingling throat syndrome.”

Acute allergic reactions have been reported in anisakidosis, when biochemical substances are released by the parasites into the flesh of the host fish. Urticaria, asthma, conjunctivitis, and contact dermatitis have been observed among workers in fish and marine products processing factories and are forms of occupational hypersensitivity.

Diagnosis

Anisakidosis should be highly suspected if there is a recent history of eating raw or improperly cooked fish or squid prior to the acute onset of symptoms. Through gastroscopic/endoscopic examination, the larvae can be visualized and removed for identification. Intestinal anisakidosis is more difficult to establish, and may be diagnosed only after surgery. Serological procedures to detect specific antibodies have been employed with good results, such as enzyme-linked immunosorbent assay (ELISA), and radioallergosorbent test (RAST).

Treatment

The main approach is to mechanically remove the larva using endoscopic forceps. It is strongly recommended that endoscopic removal be done early to avoid invasion of the gastric submucosa. Corticosteroids have been used in cases of allergic anisakidosis but clinical trials have not been performed. A possible therapeutic benefit from albendazole for intestinal anisakidosis has been reported in Spain.

Epidemiology

Human anisakidosis is not a very common infection, but it has been reported from all over the world. In Asia, the majority of reports have come from Japan and Korea, while in Europe, human cases have been identified in the Netherlands, France, Germany, Italy, Spain, and the United Kingdom. It has also been reported in North and South America.

There have been reported cases from Egypt as well. The condition is more common in the coastal population of these countries due to the consumption of raw and inadequately cooked fish. In the Philippines, anisakidosis has not yet been documented.

Considered to be high risk for anisakidosis are fish dishes such as Japanese sushi and sashimi, pickled anchovies, gravlax, salted and smoked herring, and possibly fish *bagoong* as well as fish *kinilaw* in the Philippines. Salting, marinating, pickling, smoking, and other curing techniques are effective against some foodborne pathogens, but not for anisakid larvae.

Several species of marine fish and cephalopods (squid) have been found to be infected with anisakid larvae. Mostly involved are the Pacific/Atlantic cod, Pacific halibut, red snapper, mackerel, eels, salmon, and anchovies. In the Philippines, anisakid larvae have been found in blue mackerel scad (*galunggong*), but the prevalence and density of the larvae seems to be seasonal. Infected eels (*palos*) have been found in Cebu, Mactan, and Leyte.

The increasing number of cases is believed to be due to multi-factorial causes. Deep sea marine mammals are currently being protected. Therefore, there has been an increase in the population of the definitive hosts. The worldwide distribution of the anisakid nematodes may result in widespread contamination of marine fish and squid. The increasing popularity of the consumption of sushi and sashimi globally may also contribute to the increase in cases.

Control and Prevention

In order to best control and prevent anisakidosis, marine fish, squid, and shellfish must be thoroughly cooked prior to consumption. For raw or undercooked preparations, fish and shellfish must undergo blast freezing at -35°C for at least 15 hours. Freezing at -20°C for 7 days has also been found to be effective. Furthermore, raising the

awareness of both producers and consumers of potentially infectious products through health education may be helpful.

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*Toxocara canis**Toxocara cati*

Ernesto C. Balolong, Jr., Winifreda U. de Leon

Toxocariasis is a zoonotic disease which may present as a public health problem with stray dogs and cats common in urban areas. The disease is caused by larvae of *Toxocara canis* and *Toxocara cati*, roundworms found in dogs and cats, respectively. When infective eggs of these roundworms are ingested by humans, larvae are released and penetrate the intestinal wall then migrate via the veins into the liver and the rest of the body, where they remain as larvae. *Toxocara* spp. belong to the Family Toxocaridae and Order Ascaridida.

Parasite Biology

Toxocara canis completes its life cycle in dogs (Figure 3.16). Following ingestion by the canine hosts, the larvae emerge from the eggs, penetrate the gut wall, and migrate into various tissues, where they encyst. In younger dogs, the larvae, after hatching, migrate through the circulatory system to the lungs and trachea. They eventually are coughed out, swallowed, and then develop into the adult stage in the small intestine in about 60 to 90 days after

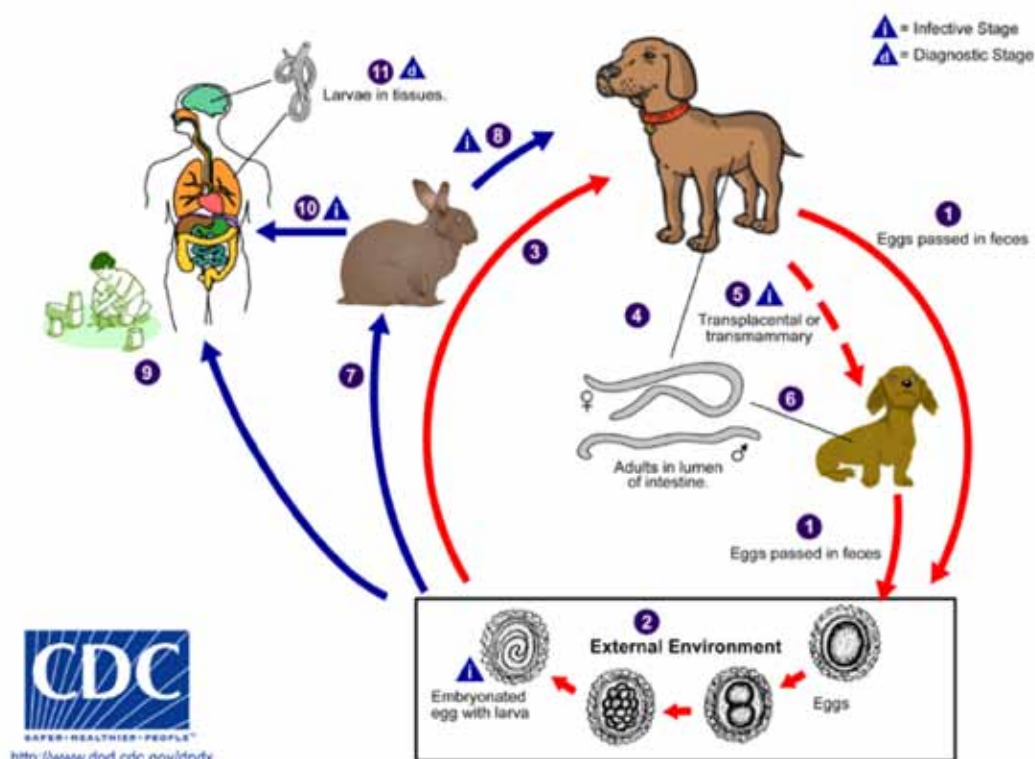


Figure 3.16. Life cycle of *Toxocara canis*
 (Accessed from www.dpd.cdc.gov/dpdx)

hatching. The female nematode produces about 200,000 eggs per day which are shed in an unembryonated form but become infective after 2 weeks to several months. These non-infective eggs need several weeks of optimal environmental conditions (10-35°C, high soil humidity) to develop into infective embryonated eggs. The embryonated eggs are resistant to freezing, moisture, and extreme pH levels for at least a year. Meanwhile in older female dogs, the encysted stages are reactivated during pregnancy, and infect their puppies through the transplacental and transmammary routes, with the adult worms establishing in the small intestine. Eggs therefore are excreted both by infected lactating females and puppies. In most adult dogs with some degree of acquired immunity, the larvae undergo larval migration to tissues and remain encysted. These encysted larvae may then be released after predation. *Toxocara canis* can also be transmitted to non-canid mammals (e.g., rabbits, chicken, cattle, sheep) or carried by earthworms, ants, and other soil-dwelling invertebrates through ingestion of organs and muscle tissue of paratenic hosts containing parasite egg or larvae.

The cat roundworm, *T. cati*, follows a life cycle similar to that of *T. canis* except that vertical transmission is attributed more to lactation than transplacental transmission. *T. cati* causes fewer cases of human infection than *T. canis*, most likely because of the defecation patterns of cats, which make environmental contamination less frequent.

Humans are accidental hosts and become infected by ingesting infective eggs from contaminated soil. After ingestion, the eggs hatch and release larvae that penetrate the intestinal wall and are carried by the circulation to different organs (e.g., liver, heart, lungs, brain, muscle, and eyes). While the larvae do not develop into adult worms in the human host, they can cause severe local reactions that may result in significant damage.

Pathogenesis and Clinical Manifestations

At least three clinical forms of TC had been reported in humans; these include visceral larva migrants (VLM), ocular larva migrants (OLM), and covert toxocariasis (CoTOX). VLM and OLM, although presented as independent clinical manifestations, can coexist.

The VLM is the result of migration and subsequent death of the larvae in the different tissues and organs, producing an intense inflammatory response manifested as eosinophilic granulomas. It is observed that the liver, lungs, central nervous system, and eyes are the most sensitive. Wheezing is a common sign of VLM, along with other lower respiratory symptoms, more commonly, bronchospasm. Progression to eosinophilic pneumonia and respiratory failure has been reported. Isolated reports describe diffused non-cavitating pulmonary nodules and pleural effusions. VLM is usually associated with liver enlargement and necrosis. Histopathology studies usually reveal granulomatous hepatitis. The spleen is enlarged less often than the liver. Generalized lymphadenopathy is an infrequent manifestation of toxocariasis. Although infrequently involved, the heart can be affected, with myocarditis as the most common problem. Loeffler endomyocarditis has also been reported.

The OLM is expressed with signs and symptoms manifested in the eyes, and occurs usually in children 5 to 10 years old. Unilateral visual impairment sometimes with strabismus is common. It is considered to be the result of a very few larvae. Occasionally, one larva is able to invade and affect almost all the ocular structures. The most serious consequence is the invasion of the retina. Other ocular lesions include posterior pole granuloma, peripheral granuloma, or a condition similar to chronic endophthalmitis. Blindness is also common.

CoTOX is the medical term used to identify a less specific syndrome where most patients are

asymptomatic and eosinophilia is less frequent. Usual symptoms may include: coughing, wheezing, chronic or recurrent abdominal pain, hepatomegaly, sleep disturbances, headache, malaise, and anorexia. Manifestations such as polyarthralgias, monoarthritis, migratory cutaneous lesions, and small-vessel vasculitis may coincide with VLM.

Another recognized syndrome is neurological toxocariasis, which is also one of the causes of encephalitis. Larvae may migrate to the brain, meninges, and may be found present in the cerebrospinal fluid (CSF). Solitary mass lesions may be observed in the brain tissue causing seizures, static encephalopathy, arachnoiditis, spinal cord lesions, optic neuritis, and eosinophilic meningitis, a form of aseptic meningitis in which the WBCs in the CSF mainly consist of eosinophils.

Diagnosis

Toxocariasis in human is difficult to diagnose because the symptoms of toxocariasis are similar to the symptoms of other infections. Fecalysis cannot be utilized in the evaluation of human toxocariasis as eggs are not produced or excreted. Definitive diagnosis of toxocariasis is based on the detection of larvae from biopsy tissues, but this test is time-consuming and difficult to perform. Currently, diagnosis is commonly based on clinical and serologic tests. Commercial immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) kits are available wherein *Toxocara* excretory-secretory (TES) antigens are used to detect IgG antibodies against the larvae. In general, however, these assays do not have adequate specificity for use in countries where other soil-transmitted helminths are endemic. Western blot is more specific but is unable to differentiate between new and old infections. Polymerase chain reaction (PCR) has good results in the identification of *Toxocara* species in tissues using animal models.

In addition to the blood test, diagnosis of toxocariasis includes identifying the presence of typical clinical signs of OLM or VLM and a history of exposure to cats and dogs.

Medical imaging techniques can be used to detect and localize granulomatous lesions due to *Toxocara* larvae. Abdominal ultrasound had shown multiple hypoechoic areas in livers of patients who initially presented with hepatomegaly, eosinophilia, and a positive *Toxocara* serology. Using computed tomography (CT), hepatic lesions appear as low-density areas. In the CNS, more sensitive magnetic resonance imaging (MRI) may reveal granulomas appearing as hyper-intense areas.

Treatment

Visceral toxocariasis can be treated with antiparasitic drugs such as albendazole or mebendazole, usually in combination with anti-inflammatory medications. Although most patients with toxocariasis recover without therapy, for those patients with neurological toxocariasis or lung or cardiac complications, anthelmintic treatment is mandatory. Patients presenting with inflammatory reaction due to higher doses of praziquantel or albendazole were found to respond very well to steroids. Treatment of ocular toxocariasis is more difficult and usually consists of measures to prevent progressive damage to the eye.

Epidemiology

Human toxocariasis is primarily a soil-transmitted zoonosis with the infection more commonly found in children than adults. Children are more at risk because of their tendency to play in soil and exhibit geophagia or soil eating, thus increasing the risk of toxocariasis. Cases are more frequently seen in children living in homes and in neighborhoods where dogs and puppies are not dewormed. Poor personal hygiene as well as consumption of inadequately washed vegetables

grown in contaminated gardens may result in chronic low-dose infections. Less commonly, zoonotic toxocariasis infection is associated with consumption of raw meat from potential paratenic hosts, such as chickens, lambs or rabbits. The seroprevalence of toxocariasis was significantly higher among persons frequently eating raw or undercooked liver than in persons who ate their meat that has been sufficiently cooked. This suggests that infective larvae can be released from animal tissues during digestion and subsequently cause human toxocariasis.

A number of surveys around the world demonstrated high rates of contamination of soil with the parasite eggs in parks, playground, and other public places (10-30%). In western countries, the prevalence of infection in dogs was reported to be about 25%, but may be as high as 30 to 60%. The prevalence of infection tends to be lower in older animals in addition to well-cared pet dogs, and higher in stray or pound dogs. This high prevalence together with the high fecundity of *Toxocara*, and the increasing number of pet animals in western countries explain the high level of soil contamination with *Toxocara* eggs. Studies have also demonstrated contamination of soil samples taken from gardens of homes where a clinical case of toxocariasis is found. *Toxocara* eggs have been recovered from salads and other raw vegetables taken from such gardens.

Prevention and Control

Toxocara control aims to prevent infection in both man and animals. Contamination of soil and environment can be greatly reduced with the control and capture of stray dogs and cats, cleaning up feces from soil and pavements, closing of potentially contaminated areas to animals and children, and implementing strategic anthelmintic treatment of dogs and cats. As dogs and cats are the sources of infection, treatment program starting at 2 to 3 weeks of age should be implemented, and repeated every 2 weeks until 12 weeks of age to

minimize environmental contamination with eggs. Adult cats and dogs should be treated every 6 months. Treatment of female dogs is also indicated after each estrus cycle.

Gardens should be fenced to prevent fecal contamination by dogs and cats. Vegetables gathered from possibly contaminated gardens should be thoroughly washed, and the consumption of raw or undercooked meat that could harbor *Toxocara* larvae should be avoided. Hand washing, especially prior to eating, should be encouraged, while hand to mouth activity should be discouraged at all times. Municipal ordinances to prevent pet dogs from entering parks and playgrounds and to require owners to remove their pets' feces from public areas should be considered.

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CHAPTER 4

Cestode Infections

Intestinal Cestodes

Winifreda U. de Leon

Taenia spp.
Taenia saginata

Taenia saginata is known as the beef tapeworm of humans. It is cosmopolitan in distribution. Humans serve only as definitive host and never as intermediate hosts. Therefore, human cysticercosis due to this species does not occur. The epidemiology, prevention, and control of *T. saginata* will be considered jointly under the section on *T. solium*.

Parasite Biology

The adult worm inhabits the upper jejunum and can live for up to 25 years. It derives nourishment from intestinal contents. Adults measure 4 to 10 m in length and may have 1,000 to 4,000 proglottids. There have been reports of worms reaching 25 m in length. The cuboidal scolex measures 1–2 mm in diameter and has four prominent acetabula (Plate 4.1).

It is devoid of hooks or a rostellum. Attached to the scolex is a short neck from which a chain of immature, mature, and gravid proglottids develop.

Mature proglottids are approximately square in shape, and they contain mature male and female reproductive organs. There are two large lobes of ovaries and a median club-shaped uterus. Follicular testes numbering 300 to 400 are scattered throughout the proglottid. The



Plate 4.1. *Taenia saginata* scolex
(Courtesy of Department of Parasitology, UP-CPH)

vagina of *T. saginata* has a sphincter. Gravid proglottids are longer than they are wide (16–20 mm by 5–7 mm) and are most distal from the neck (Plate 4.2). The uterus is distended with ova and has 15 to 20 lateral branches. The genital pores of proglottids are irregularly alternate.

Taenia spp. ova are spherical or subspherical in shape, measuring 30 to 45 μ m in diameter (Plate 4.3). The original thin outer membrane surrounding the egg is rarely retained after

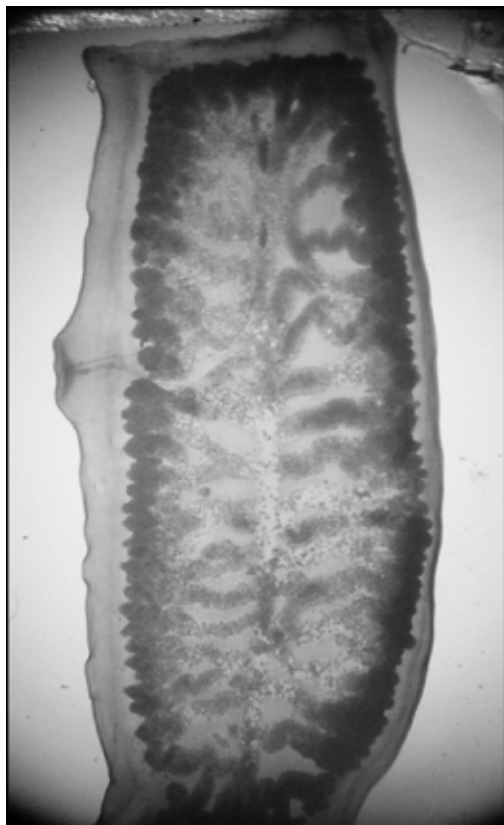


Plate 4.2. *Taenia saginata* gravid segment
(Courtesy of Department of Parasitology, UP-CPH)



Plate 4.3. *Taenia* egg
(Courtesy of Department of Parasitology, UP-CPH)

passage from the proglottid. The ova are brownish in color, with a thick embryophore which appears striated because of numerous

pits. Inside the eggshell is the oncosphere or embryo provided with three pairs of hooklets.

The gravid proglottid contains 97,000 to 124,000 ova. Annually, a worm may pass out 594,000,000 ova. Gravid proglottids undergo apolysis and are either passed out with the feces or actively crawl out of the bowel to the external environment. With apolysis of gravid segments, eggs are released and they remain viable in the soil for weeks.

Upon ingestion of the *T. saginata* eggs by cattle, the oncosphere is released. The oncosphere actively penetrates the intestinal mucosa, enters a venule, and is carried to other parts of the body. It typically enters a muscle fiber and develops into an infective stage called *Cysticercus bovis* in 2 months. The cysticercus is ovoidal, milky white, about 10 mm in diameter, and has a single scolex invaginated into a fluid-filled bladder. Humans readily become infected when these encysted larvae are ingested from raw or improperly cooked beef. The larva is digested out of the meat, and the scolex evaginates to attach to the mucosa of the small intestines where it will become mature in about 12 weeks (Figure 4.1). Usually, only one adult tapeworm is present in *T. saginata* infections. The adult seems to be irritated by alcohol, and passage of proglottids sometimes results after a drinking bout. While humans are suitable intermediate hosts for *T. solium*, they are not for *T. saginata*.

Pathogenesis and Clinical Manifestations

Among patients seen at the Department of Parasitology, College of Public Health, University of the Philippines Manila, the most common chief complaint is the passage of proglottids or segments in the stool. *T. saginata* causes mild irritation at the site of attachment. Patients with taeniasis may experience non-specific symptoms, such as epigastric pain, vague discomfort, hunger pangs, weakness, weight loss, loss of appetite, and pruritus ani (perianal itching). Rarely, entangled proglottids

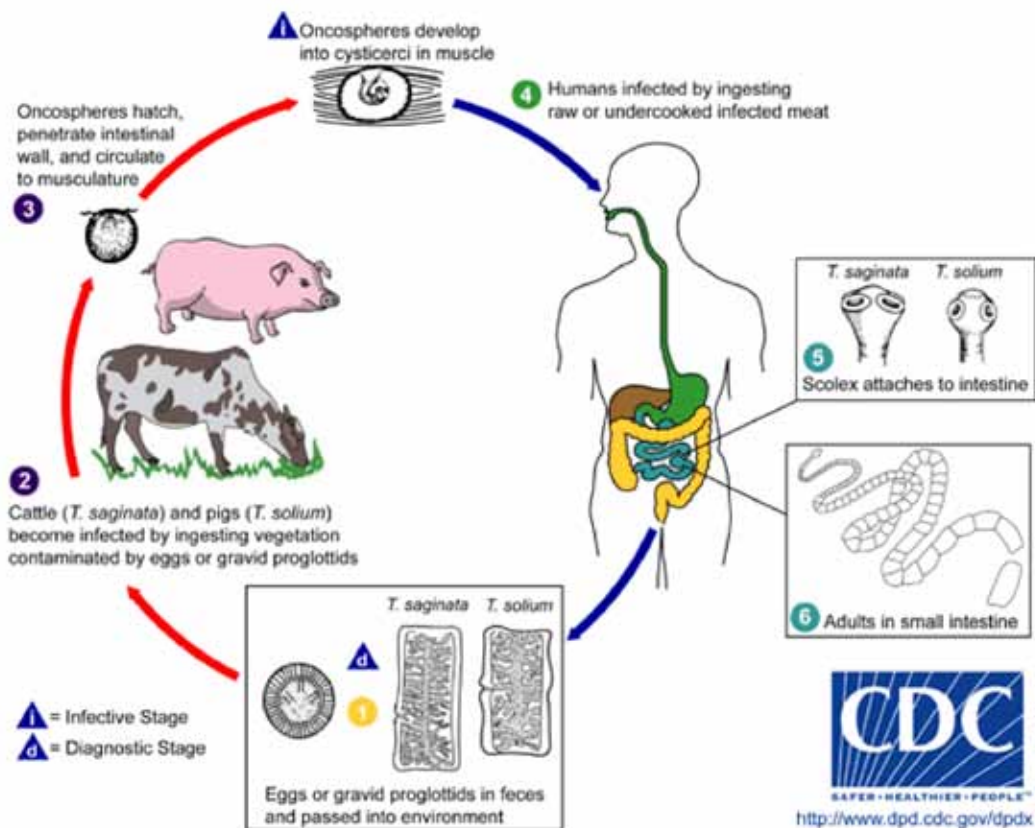


Figure 4.1. Life cycle of *Taenia* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

may result in intestinal obstruction. Individual *T. saginata* proglottids are actively motile and they have been documented to cause obstruction in the bile and pancreatic ducts, as well as the appendix. The sight of actively motile proglottids in the perianal area and in the undergarments may result in anxiety and distress.

Diagnosis

Specific diagnosis rests on identifying the characteristic proglottids, eggs or scolex. The first specimen usually brought in by patients are the gravid proglottids, either single or in chains. They are passed out with the feces or may be recovered in the patient's undergarments.

Gravid proglottids are pressed or flattened in between two glass slides and are examined against the light. This will allow one to have a rough count of the lateral branches from the main uterus. Injection of India ink through the genital pore will help one make an accurate count of the lateral branches of the uterus (15-20 for *T. saginata* and 7-13 for *T. solium*). Mature segments can be stained to demonstrate the vaginal sphincter for *T. saginata* and the accessory ovarian lobe for *T. solium*.

Examination of the stool can be done for the presence of eggs, but eggs are irregularly passed out with the stools. Concentration techniques like the formalin-ether/ethyl acetate concentration technique will be useful

in increasing the chance of demonstrating the eggs. Perianal swabs may also be useful because eggs are left in the perianal skin as the gravid segments squeeze out of the anal opening.

Treatment

The drug of choice is praziquantel. Praziquantel is given at a dose of 5 to 10 mg/kg as a single dose for both adults and children. It is not necessary to recover the scolex unless species-specific diagnosis is needed. Criteria for the cure include the following: (a) recovery of the scolex, or (b) a negative stool examination 3 months after treatment.

Taenia solium

Taenia solium is known as the pork tapeworm of man. It has a cosmopolitan distribution. Man may serve as both a definitive host and an intermediate host. Therefore, both intestinal and tissue infections occur in man.

Parasite Biology

The adult worm inhabits the upper small intestines. Like other intestinal cestodes, it derives nourishment from intestinal contents of the host. It is shorter than *T. saginata* and has less number of proglottids. The adults measure 2 to 4 m in length and may have 8,000 to 10,000 proglottids. The scolex of *T. solium* has four acetabula, but it is smaller (1 mm) and more spherical than that of the beef tapeworm (Plate 4.4). The scolex carries a cushion-like rostellum with a double crown of 25 to 30 large and small hooks, which are absent in *T. saginata*. After the scolex, comes the neck from which the proglottids develop.

The general morphology of the proglottids resembles that of *T. saginata*. The difference lies in the presence of an accessory ovarian lobe, the absence of a vaginal sphincter, and the smaller number of follicular testes (100–200) in the mature proglottid of *T. solium*. The gravid proglottid characteristically contains 7 to 13 lateral branches as opposed to 15 to 20



Plate 4.4. *Taenia solium* scolex
(Courtesy of Department of Parasitology, UP-CPH)

branches of *T. saginata*. *T. solium* proglottids are relatively less active than the proglottids of *T. saginata*. They have not been observed to actively crawl about.

The gravid proglottid contains approximately 30,000 to 50,000 ova. The gravid proglottids also undergo apolysis to eventually release eggs, which remain viable for weeks. The eggs of *T. solium* are indistinguishable from that of *T. saginata*. They measure 30 to 45 μm and have a thick brown striated embryophore surrounding a hexacanth embryo. The eggs are ingested by hogs and the oncospheres are released in the intestines (Figure 4.2).

The oncosphere penetrates the intestinal mucosa to typically encyst in muscles as cysticercus cellulosae (Plate 4.5). The cysticercus may be found in all tissues. Commonly, infected are the muscles, tongue, heart, diaphragm, liver, spleen, and mesentery. Infected meat is often called “measly pork.” Upon ingestion of improperly cooked infected meat, the larva is liberated and the scolex attaches to the intestinal mucosa. Maturity is attained in approximately 12 weeks from the time of ingestion of the cysticercus.

Man may also be an intermediate host of *T. solium*. *Taenia* eggs are very resistant and when

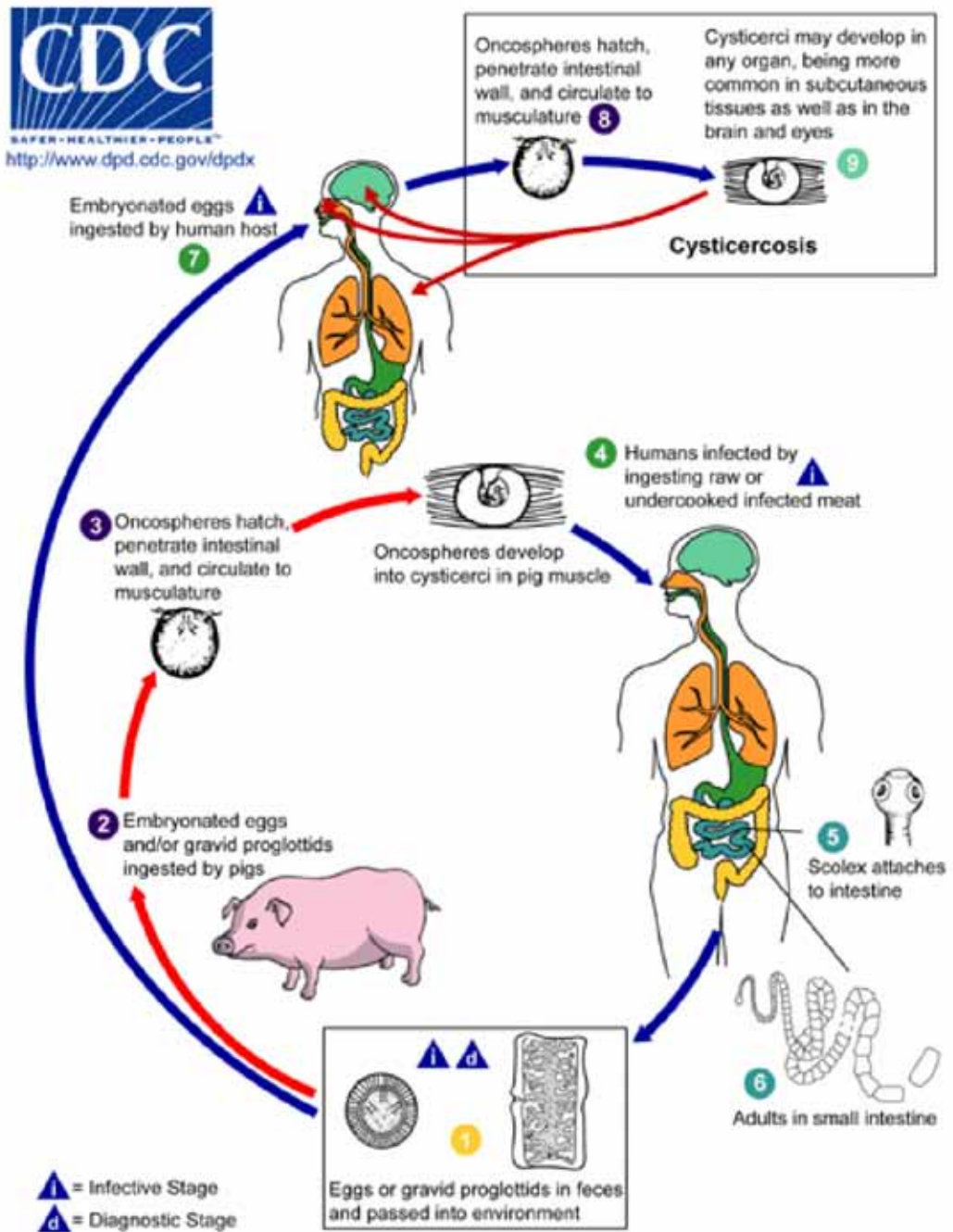


Figure 4.2. Life cycle of *Taenia solium* (cysticercosis)
(Accessed from www.dpd.cdc.gov/dpdx)

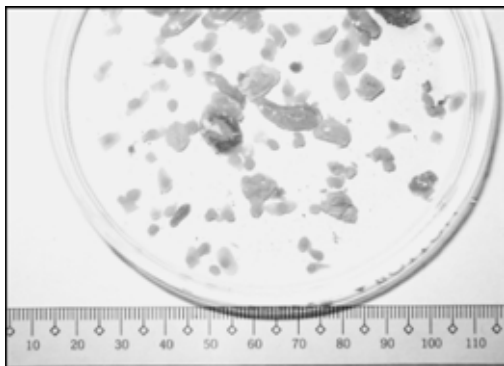


Plate 4.5. *Cysticercus cellulosae* from pork
(Courtesy of the Department of Parasitology,
UP-CPH)

the eggs are ingested, development to cysticerci ensues as it does in pigs. The oncosphere hatches in the duodenum, and spreads to different organs through the bloodstream. This results in human cysticercosis. The mature cysticercus is oval, translucent, and has an opaque invaginated scolex with four suckers and a circlet of hooks. It is usually encapsulated with adventitious host tissue. However, in the vitreous humor and in the brain, it may be unencapsulated. A full size of 5 mm may be attained in 10 weeks.

Human infection with *cysticercus cellulosae* can be acquired through fecal-oral route by ingesting *Taenia solium* eggs from contaminated food or drink. Individuals harboring the adult *Taenia solium* can infect themselves (autoinfection) due to poor hygienic practice.

Pathogenesis and Clinical Manifestations

A. Intestinal infection

T. solium intestinal infection results in mild non-specific abdominal complaints. Unlike in *T. saginata* infections, proglottids are not as active and, therefore, obstruction of the bile duct, pancreatic duct, or the appendix is unlikely.

B. Cysticercosis

The cysticerci are often multiple and can develop in any organ or tissue. Most commonly,

they are located in striated muscle and in the brain, but the subcutaneous tissues, eye, heart, lung, and peritoneum may be involved. The living cyst may produce inflammation. Cysts may survive up to 5 years. Upon death, cystic fluid increases and there is a pronounced tissue response to the parasite. The parasite is eventually calcified.

Symptomatology is dependent on the number, size, and location of the lesion. One of the most serious manifestations is neurocysticercosis (NCC), which is considered as one of the most serious zoonotic diseases worldwide. Cysticerci containing a scolex may be found in the brain parenchyma or floating freely in the ventricles. Cysticerci may also appear as large vesicular structures devoid of a scolex and are usually located in the basal cisternal spaces. NCC is divided into two general forms, parenchymal and extraparenchymal, which, in turn, is further divided into subarachnoid or meningitic, intraventricular, and spinal. Clinical manifestations and corresponding management depend on the form of NCC present in the patient. Focal neurologic deficits are usually encountered in parenchymal NCC. They would depend on the location of the cysts. Focal or generalized seizures are observed when cysts are located in the cortex. The subarachnoid form may lead to an aggressive form of NCC called racemous cysticercosis. In this form, there is a proliferation of cysts in the base of the brain. This form has a poor prognosis. In the intraventricular form, cysts are usually present in the third or fourth ventricle and often lead to obstructive hydrocephalus. The spinal form is rare.

The death of the larva leads to inflammation of the affected region. Calcification is the end-result of the cellular reaction. Convulsions are the most common manifestations of cerebral cysticercosis. Visual and motor deficits, headache, and vomiting may occur. Cerebrospinal fluid (CSF) tap results may show an increased opening pressure, elevated protein,

decreased glucose, and increased mononuclear cells. Half of the cases may present with CSF eosinophilia without peripheral blood eosinophilia.

In the eyes, cysticerci are often retinal or subretinal in location. They may float freely in the vitreous or aqueous humors. Vision is usually affected due to chorioretinitis and vasculitis. Detachment of the retina has also been reported. The patient may complain of intraorbital pain, photopsia, and blurring or loss of vision.

Diagnosis

A. *Taeniasis*

Specific diagnosis of taeniasis rests on identifying the characteristic proglottids, eggs, or scolex as described in the *T. saginata* subsection.

B. *Cysticercosis*

Neurocysticercosis may be suspected in a patient coming from an endemic area with epileptic seizures without associated systemic symptoms. Concomitant infection with *T. solium* adult occurs only in 25% of cases. If a patient has subcutaneous cysticerci concomitant with neurologic symptoms, this provides presumptive evidence for neurocysticercosis. CSF abnormalities such as an elevated protein, reduced glucose, and increased mononuclear cells may be seen. Computed axial tomography (CAT) scans and nuclear magnetic resonance imaging (MRI) are useful for localizing cysticerci and evaluating the pathology before and after treatment.

There are three main CAT scan patterns: (a) a round low-density area without surrounding enhancement after administration of contrast dye, (b) ring-like enhancement after injection of contrast dye, and (c) a small calcified area within a cystic space. The first pattern shows a viable larva with no inflammation; the second, a dead larva; and the third shows a dead scolex.

Ophthalmic cysticercosis can be diagnosed through the visualization of the cysticerci using ophthalmoscopy but the procedure may induce movement and/or evagination of the scolex. Muscular and subcutaneous cysticerci are usually palpable and can be recovered through tissue biopsy for histopathologic processing.

Serologic tests include serum and CSF enzyme-linked immunosorbent assay (ELISA) and electro-immuno transfer blot (EITB) or Western blot for specific IgG and IgM anticysticercal antibodies. These tests have a sensitivity of 75 to 100% using a partially purified glycoprotein antigen to detect antibodies. Dot-ELISA test is a very good screening test for cysticercosis. It uses crude antigen from the cysticerci obtained from pigs. Recent studies are looking into the use of antigen B of cysticercus cellulosae as a useful adjunct in diagnosis.

Treatment

A. *Taeniasis*

The drugs of choice are praziquantel and niclosamide. Because of the theoretical possibility of autoinfection and subsequent cysticercosis, treatment should not be delayed. Praziquantel is given as 5 to 10 mg/kg, single dose for both adults and children. Niclosamide is not available locally. Criteria for cure include the following: (a) recovery of the scolex, or (b) a negative stool examination 3 months after treatment.

B. *Cysticercosis*

Management of NCC depends on the form present in the patient. Multiple parenchymal cystic lesions are treated by giving praziquantel at a dose of 50 to 75 mg/kg divided into three doses for 30 days or albendazole at a dose of 400 mg twice daily for 8 to 30 days. Corticosteroids are then given (either 80 mg of prednisone or 10 mg of IM dexamethasone) 4 hours after the last dose. Parenchymal

forms presenting as cysticercotic encephalitis or those with massive parasitic infection are given high dose corticosteroid therapy and mannitol in cases of increased intracranial pressure. Many experts do not recommend giving praziquantel or albendazole in these cases. For the subarachnoid form, some experts recommend surgical removal of the lesions, while others recommend albendazole therapy in which albendazole is given at a dose of 10 to 15 mg/kg/day for 8 days. Although albendazole therapy has been shown to have several benefits, there are reports of associated meningeal fibrosis and hydrocephalus. Ventricular forms are best treated with surgical removal of the cyst.

Ocular cysticercosis should be treated surgically before praziquantel or albendazole is given because ocular inflammation cannot be controlled with steroids. Symptomatic cysts outside the CNS may be surgically removed.

Epidemiology

The distribution of *T. solium* and *T. saginata* infections is highly related to the habit of eating raw or improperly cooked meat. Abstinence from beef as part of the religious beliefs among the Hindus prevent *T. saginata* infections, while among the Moslems, prevention of *T. solium* infections happens because of abstinence from pork. Both tapeworms have a cosmopolitan distribution, although *T. solium* is especially common in Slavic countries, Latin America, Southeast Asia, China, and India. *T. saginata* has high endemicity in Ethiopia and East Africa. It has also been reported in Japan, Europe, Australia, Canada, and the United States of America.

Maintenance of the life cycle in nature is dependent on the level of environmental sanitation practiced in the area. Animal intermediate hosts, especially pigs should be kept in pens to avoid access to human feces. Contamination of the grazing fields with human feces favors infection of the intermediate hosts.

Taenia asiatica

Taenia asiatica, a third *Taenia* species, has been reported in Taiwan, Korea, Thailand, and Indonesia. This parasite was initially believed to be closely related to *Taenia saginata*. In contrast to *Taenia saginata*, however, the cysticercus larvae of *Taenia asiatica* were found in the liver of variable intermediate hosts that include pigs, cattle, goats, wild boars, and monkeys, hence the term cysticercus viscerotropica. The cysticercus has wart-like protuberances on the external surface and contains an invaginated scolex armed with vestigial hooklets.

The length of the adult may vary between 4 to 8 m with 300 to 1,000 segments. Similar to *Taenia saginata*, the scolex is devoid of hooklets but there is a prominent rostellum. The gravid proglottids have posterior protuberance with 11 to 32 lateral branches arising from the main uterus. The mature segments, on the other hand, were found to carry a vaginal sphincter. Due to the number of uterine branches and the presence of vaginal sphincter, *Taenia asiatica* may be misidentified as *Taenia saginata*.

A collaborative work with Japanese scientists was undertaken by the College of Public Health, University of the Philippines Manila. Gravid segments from six patients, identified earlier as *Taenia saginata* were subjected to genetic studies and the mitochondrial RNA of five out of the six samples were found compatible with *Taenia asiatica*. Further studies of this kind will establish the prevalence and the magnitude of the problem regarding this parasite.

In the Philippines, *T. saginata* infection is more common than *T. solium* infection. Surveys of animal intermediate hosts however showed that pigs are infected more than cows or cattle. The overall prevalence of taeniasis is only 0.56% in selected areas. In isolated foci, a prevalence of 11 to 15% for *T. saginata* has been reported. Many of the identified cases were adult males who came from the Northern Luzon provinces, where eating raw or undercooked meat while

drinking alcohol is a delicacy. Neurocysticercosis has been reported in local literature. There has been one report of ocular cysticercosis.

Prevention and Control

Prevention and control of taeniasis may appear simple but may be difficult to implement. Thorough cooking of meat is a primary measure. Freezing at -20°C for 10 days kills the cysticerci. Sanitary inspection of all slaughtered pigs, cows, and cattle should be done. Meat inspection should include examination of the liver as well.

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Hymenolepis nana

Hymenolepis nana, commonly known as the dwarf tapeworm, is a cyclophyllidean tapeworm and is the smallest tapeworm infecting humans. It is found worldwide, mainly among children. The parasite is the only human tapeworm, which can complete its entire life cycle in a single host, indicating that it does not require an obligatory intermediate host. Man can harbor both the adult and the larval stages of the parasite.

Parasite Biology

The adults, with a delicate strobila measuring from 25 to 45 mm in length and 1 mm in width, reside in the ileum. The scolex is subglobular with four cup-shaped suckers (Plate 4.6). There is a retractable rostellum armed with a single row of 20 to 30 Y-shaped hooklets. The neck is long and slender. The anterior proglottids are short and the posterior ones are broader than long. No more than 175 to 220 segments compose the entire length of the strobila. The proglottids measure 0.15 to 0.3 mm in length and 0.8 to 1.0 mm in width. The genital pores are found along the same side of the segments.



Plate 4.6. *Hymenolepis* spp. scolex
(Courtesy of the Department of Parasitology,
UP-CPH)

Mature proglottids contain three ovoid testes and one ovary in a more or less straight pattern across the segment. When segments become gravid, the testes and the ovary disappear while the uterus hollows out and becomes filled with eggs. Gravid segments (Plate 4.7) are separated from the strobila and disintegrate as they pass out of the intestines, releasing eggs in the stool.

Eggs are spherical or subspherical, colorless or clay-colored, measuring 30 to 47 μm in diameter (Plate 4.8). The oncosphere has a thin outer membrane and a thick inner membrane with conspicuous bipolar thickenings, from each of which arise four to eight hair-like polar filaments embedded in the inner membrane. These eggs, however, die immediately once passed out into the environment.

The life cycle has a dual pathway: a direct and an indirect development (Figure 4.3). In the direct cycle, the host ingests eggs, which hatch in the duodenum. The liberated embryos penetrate the mucosal villi and develop into the

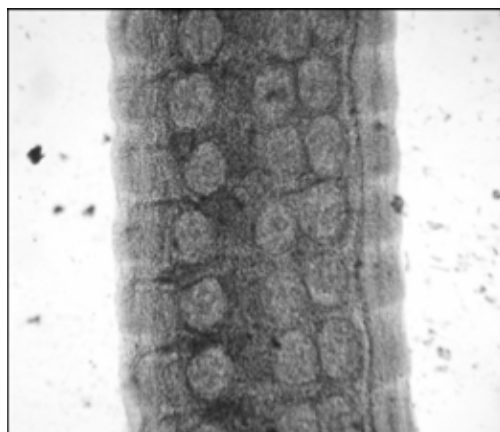


Plate 4.7. *Hymenolepis* spp. gravid segment
(Courtesy of the Department of Parasitology,
UP-CPH)

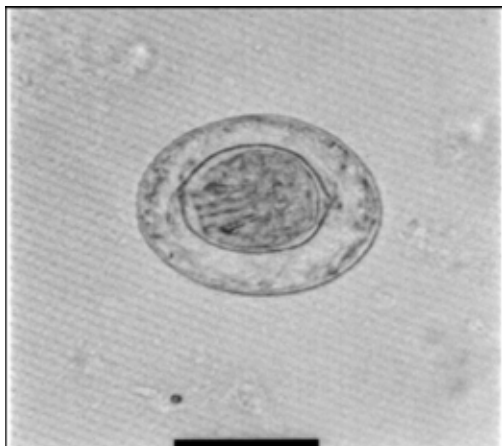


Plate 4.8. *Hymenolepis nana* egg
(From World Health Organization. Bench Aids for the diagnosis of intestinal parasites. Geneva, Switzerland: WHO Publications; 1994.)

infective cysticeroid larvae. After 4 to 5 days, the larvae break out of the villi and attach to the intestinal mucosa to develop into adults. Infection through the indirect cycle is usually via the accidental ingestion of infected arthropod intermediate hosts like the rice and flour beetles (*Tenebrio* sp.) and sometimes through fomites, water, or food contaminated with the larvae. The cysticeroid larvae are released and will eventually develop into the adult tapeworms in the intestines of the host. It takes 20 to 30 days from the time of ingestion for the eggs to appear in the feces. Eggs are optimally viable immediately after discharge from the bowel. Autoinfection can occur through the fecal-oral route or within the small bowel. Oncospheres from the eggs are released and they invade the host villi to start a new generation.

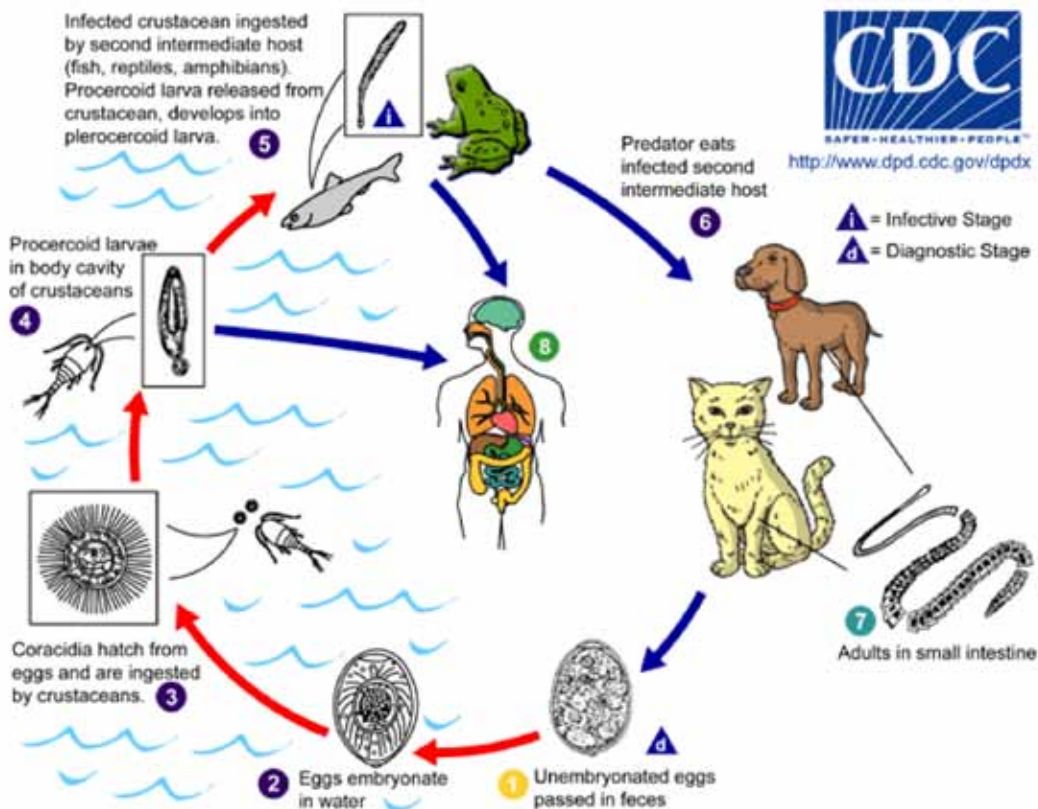


Figure 4.3. Life cycle of *Hymenolepis nana*
(Accessed from www.dpd.cdc.gov/dpdx)

Pathogenesis and Clinical Manifestations

Symptoms are generally produced because of the patient's immunological response to the parasite. Light worm burden is generally asymptomatic. Clinical manifestations include headache, dizziness, anorexia, pruritus of nose and anus, diarrhea, vomiting, abdominal pain, pallor, and weight loss. Some infected children are restless, irritable, and exhibit sleep disturbances. Rarely, convulsions occur. Heavy infections may result in enteritis due to necrosis and desquamation of the intestinal epithelial cells. With time, regulatory immunity may limit or eventually clear the *H. nana* population spontaneously. Infections in children resolve spontaneously in adolescence.

Diagnosis

Specific diagnosis is made by demonstration of the characteristic eggs in the patient's stool. In light infections, concentration of the stool specimens on alternate days is useful. Generally, proglottids are not recovered because they undergo degeneration prior to passage with stools.

Treatment

The drug of choice is praziquantel given as a 25 mg/kg single dose. Praziquantel causes vacuolization and disruption of the tegument in the neck region. The drug dosage for hymenolepiasis is higher than that for taeniasis because of the relatively resistant cysticercoids in the intestinal tissue. Stool examination may be repeated after 2 weeks. Treatment is usually repeated after 2 weeks to cover for the worms emerging from the remaining viable cysticercoids. Treatment is considered successful if stools are negative for *H. nana* eggs at one month post treatment. Nitazoxanide (500 mg orally for 3 days) may be used as an alternative drug.

Evidence in mice has shown that infection is influenced by steroid treatment or by T-cell deprivation allowing an increased multiplication

of abnormal cysticercoids in the viscera that occurs in an immunosuppressed condition. This may suggest that the parasitic condition should be treated first before any immunosuppressive therapy is given.

Epidemiology

Hymenolepis nana is found in areas with warm climate like Southern USA, Latin America, the Mediterranean, East Asia, and the Philippines. An estimated 20 million people are infected. Transmission generally occurs where there is poor sanitation, overcrowding, and poor personal hygiene practices. Direct contact plays an important role because the eggs cannot survive long outside the host. It is a familial and institutional infection common in orphanages, day care centers, and mental institutions. Prevalence varies from 5 to 20% among children and young adults in communities where direct transfer of embryonated eggs from hand to mouth is likely to occur.

This human tapeworm is also found among the mice and less frequently among the rats. The species in mice and rats is considered to be a distinct subspecies called *H. nana* var. *fraterna*. Although very rare, some strains were found to be infectious to humans as well. Therefore, infected mice and rats may be potential sources of infection.

In the Philippines, two independent surveys of Jueco in 1983 and Cross, et al. in 1984 showed a prevalence of less than 1% in humans. Infection among rodents was found to be low as well.

Prevention and Control

The life cycle involves a single host and transmission is direct. This makes prevention more difficult, especially in crowded dwellings. Emphasis should be placed on personal hygiene and environmental sanitation. Infected cases should be thoroughly treated. Rodent control must be observed. Food must be properly stored and protected from possible infestation with grain beetles.

Hymenolepis diminuta

Hymenolepis diminuta is a cosmopolitan parasite primarily of rats, hence the common name, rat tapeworm. Accidental human infections do occur resulting in hymenolepiasis. Aside from morphological differences with *H. nana*, *H. diminuta* differs in that it requires an intermediate host.

Parasite Biology

The adult tapeworm is larger than *H. nana*. The worm measures about 60 cm in length. The scolex differs from that of the *H. nana* by having a rudimentary unarmed rostellum. As in *H. nana*, mature proglottids are broader than they are long, and the arrangement and number of sexual organs are similar: three ovoid testes and one ovary in a more or less straight pattern across the segment. The proglottids are larger and may reach 0.75 mm in length and 3.5 mm in width. The genital pores are unilateral. Each gravid proglottid contains a sac-like uterus filled with eggs.

H. diminuta eggs are circular, about 60 to 80 μm in diameter and are bile-stained (Plate 4.9). The oncosphere is enclosed in an inner membrane, which has bipolar thickenings but lacks the bipolar filaments. The hooklets usually have a fan-like arrangement.

The gravid proglottids separate from the main body of the worm, disintegrate, and release eggs into the feces. Eggs, when ingested by a wide range of adult and larval insects like fleas, beetles, cockroaches, mealworms, and earwigs, develop into the infective cysticercoid larvae. When these infected insects are ingested by the rat or accidentally ingested by man, the larva is released and develops into the adult worm in about three weeks (Figure 4.4).

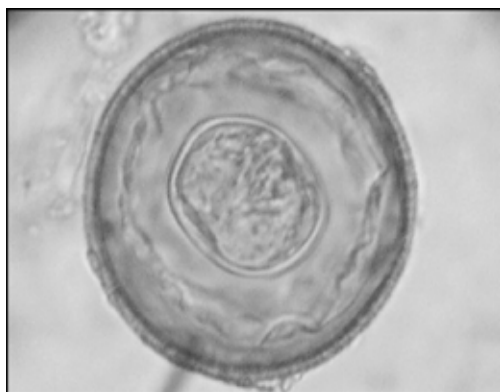


Plate 4.9. *Hymenolepis diminuta* egg
(Courtesy of the Department of Parasitology,
UP-CPH)

Pathogenesis and Clinical Manifestations

The worm burden in rodents is relatively low. In man, the highest number recorded is 19 worms. Clinical manifestations are minimal and non-specific. The life span of *H. diminuta* in humans is short, which possibly explains why human infections are usually light.

Diagnosis

Diagnosis is based on the identification of eggs from the stool. *H. diminuta* eggs are distinguished from *H. nana* eggs by their more circular shape, larger size, and lack of bipolar filaments. At times, the whole worm is expelled and the morphology of the scolex may be used as an aid in diagnosis.

Treatment

Treatment is similar to *Hymenolepis nana*. Praziquantel is given as a 25 mg/kg single dose.

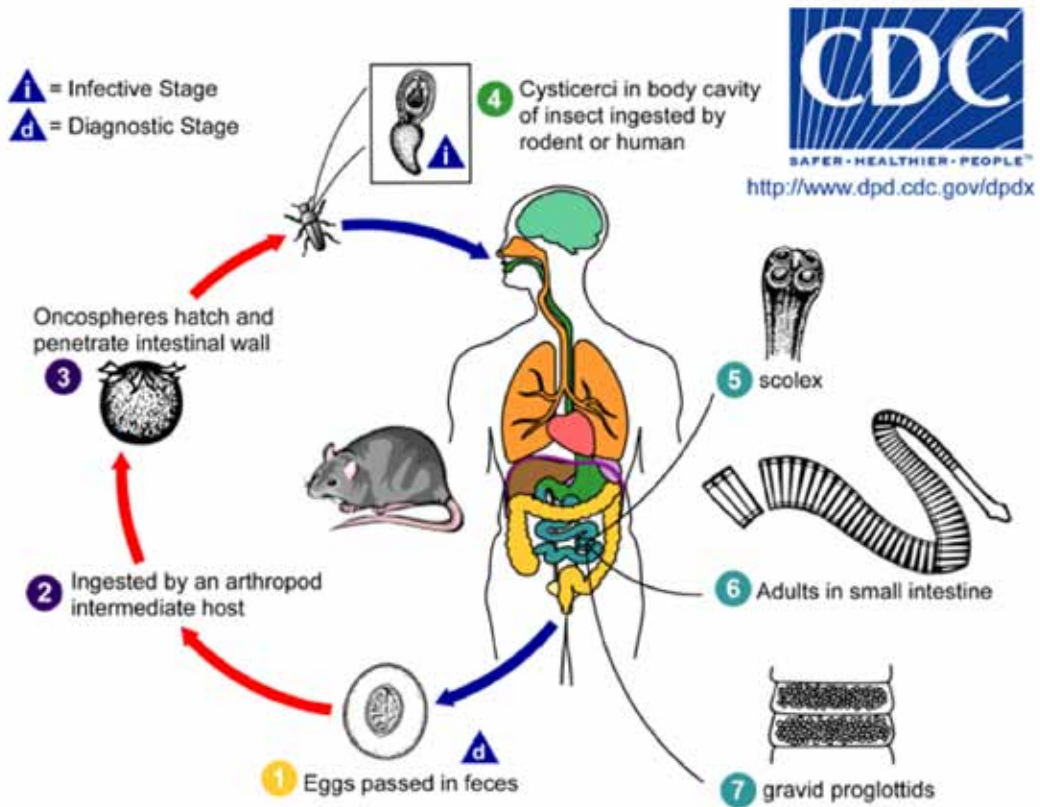


Figure 4.4. Life cycle of *Hymenolepis diminuta*
 (Accessed from www.dpd.cdc.gov/dpdx)

Epidemiology

Human infection occurs worldwide but is more common among children than adults in poor communities with rat infestation. It probably occurs by accidental ingestion of grain beetles infesting dried grains, dried fruits, flour, and cereals. In a nationwide survey of rats in the Philippines, prevalence of *H. diminuta* was found to be about 8%.

Prevention and Control

Prevention and control measures include rodent control, elimination of the insect intermediate hosts, protection of food, especially the precooked cereals from such insects, sanitary disposal of human waste, and treatment of human cases.

Dipylidium caninum

Dipylidium caninum is a very common intestinal parasite of dogs and cats worldwide, especially in dog populations where ectoparasitism is high. Dipylidiasis in humans is accidental and is observed to be more common in children than in adults.

Parasite Biology

The pale reddish adult worm measures 10 to 70 cm in length. The scolex is small and globular with four deeply cupped suckers and a protrusible rostellum, which is armed with one to seven rows of rose thorn-shaped hooklets. The proglottids are narrow with two sets of male and female reproductive organs and bilateral genital pores, earning for this parasite the common name double-pored tapeworm. The gravid proglottids have the size and shape of a pumpkin seed and are filled with capsules or packets of about 8 to 15 eggs enclosed in an embryonic membrane (Plate 4.10). When the gravid segments are detached, they either migrate out of the anus or are passed out with the feces. The ova are released by contraction of the proglottid or by its disintegration outside the host. Eggs are spherical, thin-shelled with a hexacanth embryo (Plate 4.11).

Some of the egg capsules may remain in the fur of the host or in the host's resting place. Here, larval fleas ingest the ova as they feed on epidermal debris. Among the intermediate hosts are the larval stages of *Ctenocephalides canis* (dog flea), *Ctenocephalides felis* (cat flea), and/or *Pulex irritans* (human flea). *Trichodectes canis* (dog louse) has also been involved. In the body cavity of the arthropod, the hexacanth embryo develops into the cysticercoid larvae, which is able to survive the flea's development. When the insect is ingested by mammalian hosts (dogs, cats, humans), the cysticercoid is liberated and becomes an adult in 3 to 4 weeks (Figure 4.5).

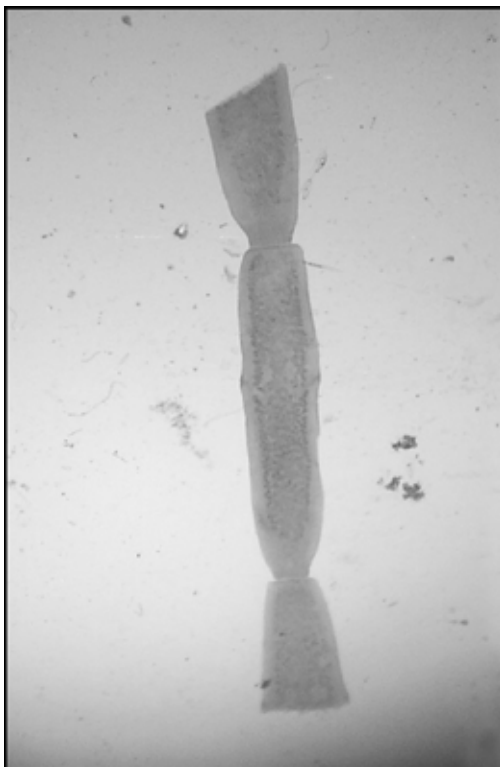


Plate 4.10. *Dipylidium caninum* gravid segment
(Courtesy of the Department of Parasitology,
UP-CPH)

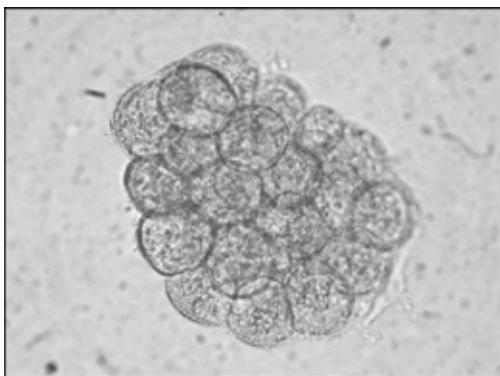


Plate 4.11. *Dipylidium caninum* egg capsule
(Courtesy of the Department of Parasitology,
UP-CPH)

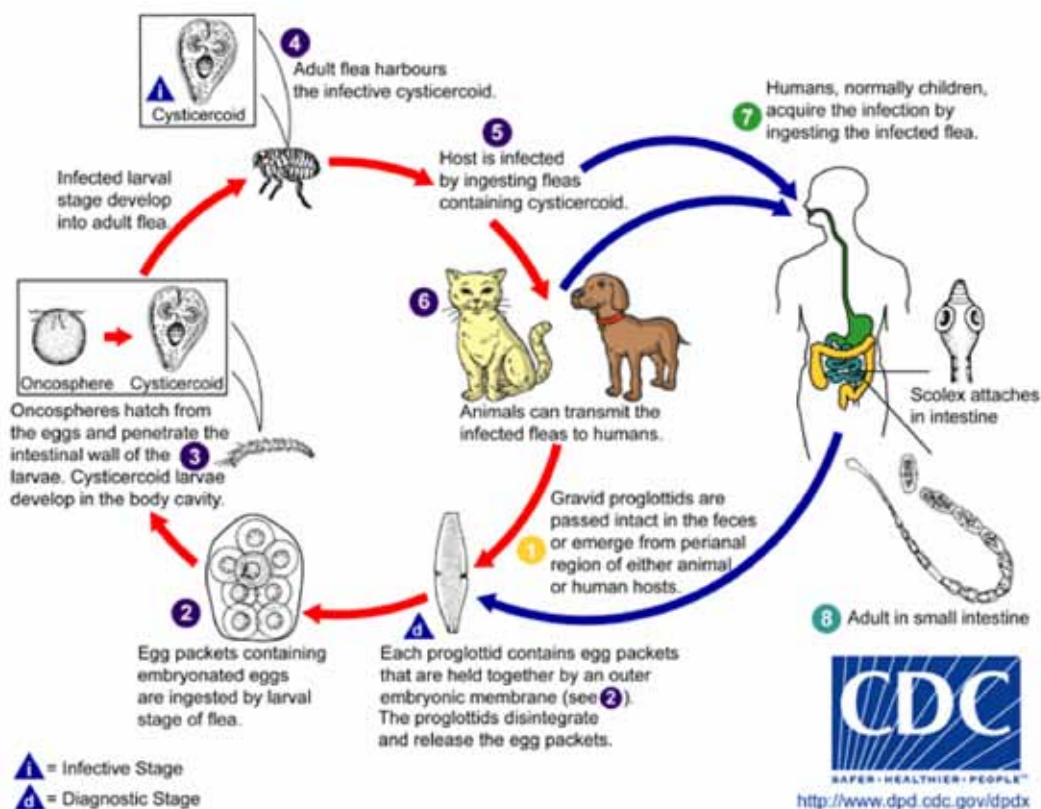


Figure 4.5. Life cycle of *Dipylidium caninum*
(Accessed from www.dpd.cdc.gov/dpdx)

Pathogenesis and Clinical Manifestations

Infection is rarely heavy and symptoms are minimal. Slight intestinal discomfort, epigastric pain, diarrhea, anal pruritus, and allergic reactions have been reported. While most patients are asymptomatic, moderate eosinophilia has been reported.

Diagnosis

Diagnosis is established upon recovery of the characteristic gravid proglottids passed out singly or in chain. Gravid proglottids may crawl out of the anus, and may be passed out involuntarily. Proglottids should be pressed or flattened between two glass slides for examination. Stool examination for the presence

of the egg capsules is not recommended, since the gravid proglottids do not disintegrate in the intestines but in the environment. Egg capsules are rarely recovered from the stool.

Treatment

Treatment consists of praziquantel 5 to 10 mg/kg given as a single dose.

Epidemiology

Human infection is rare but has been reported in European countries, USA, Argentina, Rhodesia, China, and the Philippines. Infants and very young children are usually infected because of their close contact with their pet cats and dogs. Likely, transmission could have

occurred through hand to mouth contamination or accidentally swallowing the arthropod hosts when hugging and kissing the animal. Parents usually observe the presence of actively motile proglottids in children feces or underwear. Adults are not commonly infected possibly because of age tolerance against the parasite.

In the Philippines, the first human infection was reported as early as 1912 by M.P. Mendoza-Guanzon in a child. Surveys of dogs in the

city of Manila showed a prevalence of 5.19 to 36.0%, while dissection of dog and cat fleas for cysticercoids showed only a prevalence of 2.4%.

Prevention and Control

Periodic deworming of pet cats and dogs is recommended. Insecticide dusting of dogs and cats are effective against fleas. The potential danger of playing with pets must be included in the health education of children.

Raillietina garrisoni

Raillietina garrisoni belongs to the Family Davaineidae. *Raillietina madagascariensis* was first reported by Garrison to be present in an adult Filipino in 1911. *R. garrisoni* was later documented in three children. It is generally believed that the species are identical. Tubangui further showed that this was a common tapeworm of rats. Almost all human infections in the Philippines have involved children.

Parasite Biology

The tapeworm (Plate 4.12) is about 60 cm in length with a minute, subglobular scolex with four acetabula. The rostellum is armed with two alternating circular rows of 90 to 140 hammer-shaped hooks. Several rows of spines also surround the rostellum. The mature proglottid has a bilobed ovary surrounded by 36 to 50 ovoid testes. The genital pore opens on the side near the anterior lateral border of the segment. The fully gravid proglottids are about 2 mm in length containing 200 to 400 egg capsules with one to four spindle-shaped eggs. The oncosphere is enclosed in two thin

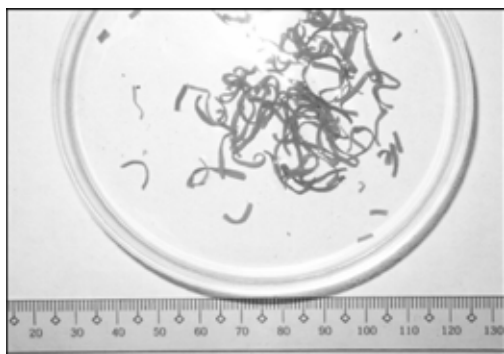


Plate 4.12. *Raillietina garrisoni* adult
(Courtesy of the Department of Parasitology,
UP-CPH)

membranes: an outer elongated membrane and an inner spherical membrane. The gravid segments detach from the rest of the strobila by apolysis and may be passed out in the feces. The segments are motile, white, and appear like grains of rice when passed out with the feces.

Gravid segments may be ingested by the insect intermediate host, the flour beetle *Tribolium confusum* (Plate 4.13). The development from egg to the cysticercoid larval stage takes about two weeks. Infected insects are accidentally ingested and the cysticercoid larva attaches to the intestinal villi to develop into an adult in about 8 weeks. Direct infection does not occur if eggs are ingested by the mammalian host; therefore, there is no autoinfection in *R. garrisoni* infection.

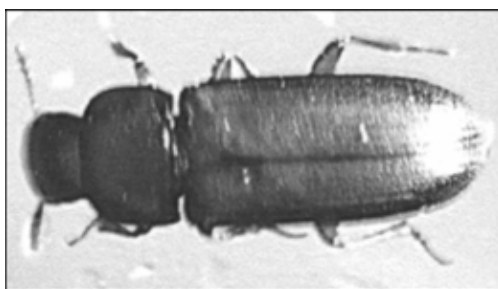


Plate 4.13. Flour beetle (*Tribolium* spp.), the
intermediate host of *Raillietina garrisoni*
(Courtesy of Dr. Lilian de las Llagas)

Pathogenesis and Clinical Manifestations

Patients are usually asymptomatic. Children are brought for medical consultation when proglottids are passed out with their feces.

Diagnosis

Diagnosis is made by finding the characteristic proglottids or ova in stools.

Treatment

Sometimes, long strobila or the complete tapeworm may be expelled by the child spontaneously without treatment. Praziquantel may be given to expel the worm.

Epidemiology

Raillietina garrisoni is a common intestinal cestode of rodents in the Philippines. More than 20 human infections have been reported in Philippine scientific journals. Almost all infections occurred in children who were below three years of age. In Thailand, the first human case was reported as early as 1891, and another 11 cases, all children, were reported from 1960 to 1970. *Raillietina* infections have also been reported in Tokyo, Taiwan, Australia, Ecuador, and North Iran. In all cases, the infections were confined to children usually 5 years and below.

Prevention and Control

Elimination of rodents from households, proper storage of grain products, and sanitary waste disposal can help preventive infection.

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Diphyllobothrium latum

Diphyllobothrium latum belongs to the Order Pseudophyllidea. It is just one of the 13 species of *Diphyllobothrium* that infects human. It is commonly called the fish tapeworm or the broad tapeworm. Diphyllbothriasis refers to the intestinal infection with the adult worm.

Parasite Biology

The adult tapeworm measures from 3 to 10 m in length and may have 4,000 proglottids. The scolex is spatulate and measures 2 to 3 mm in length by 1 mm in diameter (Plate 4.14). It has two bothria or sucking grooves, which are located dorsally and ventrally. The neck is long and attenuated, and is followed by immature proglottids. The terminal four-fifths of the worm is composed of mature and gravid proglottids. The mature proglottid has a longer width than its length. It measures 2 to 4 mm in length by 10 to 12 mm in width, and contains one set of reproductive organs. The testes are located in the dorsolateral part of the proglottid. The vas efferens converge to form a vas deferens and this enlarges into a seminal vesicle and terminates in a muscular cirrus

found at the midventral common genital pore. The dark, rosette-like, coiled uterus located in the middle of the gravid proglottid extends from the ootype and opens through a uterine pore in the midventral line behind the common genital pore. A symmetrical bilobed ovary is present at the posterior third of the proglottid immediately above the Mehli's gland. From the common genital pore, the vagina extends up to join the oviduct and the vitelline duct. Unlike in Taeniidae, the proglottids of *D. latum* disintegrate only when the segment has completed its reproductive function.

With distention of the uterus, the uterine pore is relaxed and unembryonated ova are discharged from the proglottid. Approximately 1,000,000 ova may be released daily. The ova (Plate 4.15) are usually yellowish brown, with a moderately thick shell and an inconspicuous operculum. Opposite the operculum is a small knob-like thickening. The mean size of the eggs is 66 by 44 μm , with a range of 58 to 76 μm in length and 40 to 51 μm in width.

The ova complete their development in water and release the free-swimming coracidium



Plate 4.14. *Diphyllobothrium latum* scolex
(Courtesy of the Department of Parasitology,
UP-CPH)



Plate 4.15. *Diphyllobothrium latum* egg
(Courtesy of the Department of Parasitology,
UP-CPH)

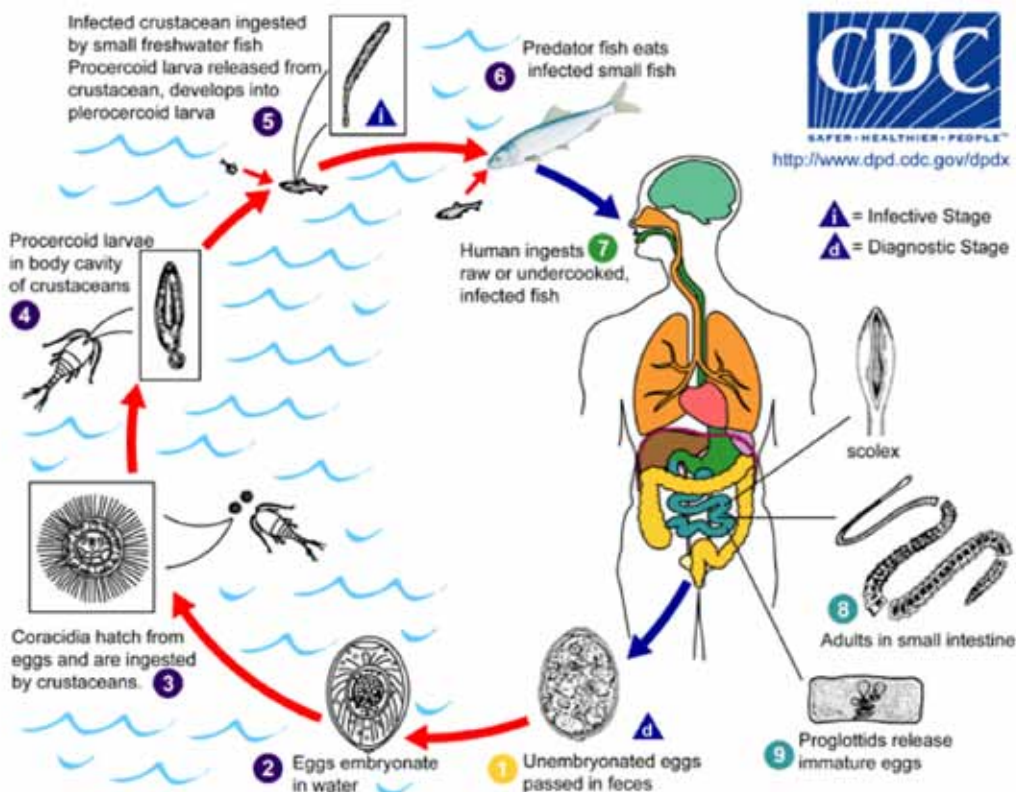


Figure 4.6. Life cycle of *Diphyllobothrium latum*
(Accessed from www.dpd.cdc.gov/dpdx)

(Figure 4.6), a ciliated embryo, which is ingested by freshwater copepods of the genera *Cyclops* and *Diaptomus*. A proceroid larva develops in the copepod. The proceroid measures 550 μm and still retains the three hooklets in the cercomer, a caudal attachment organ. The copepod is in turn ingested by fish. The proceroid larva migrates through fish tissues and develops into a plerocercoid larva in the muscles and viscera. The plerocercoid larva or sparganum measures 20 mm or more and appears glistening, opaque white, and unsegmented. Fish with the infective plerocercoid larva is ingested raw by a definitive host like man, dog, cat, and other mammals. Carnivorous fish may serve as paratenic or transport hosts as well. Among fish intermediate hosts are perch, trout, salmon, and pike. In the definitive host, the plerocercoid attaches to the

intestinal wall and reaches maturity in about 3 weeks.

Pathogenesis and Clinical Manifestations

Infections are usually limited to one worm, although there have been reports of mechanical obstruction due to a large number of worms. Infected individuals may show no signs of disease. Some, however, may experience nervous disturbances, digestive disorders, abdominal discomfort, weight loss, weakness, and anemia. Symptoms may be due to absorbed toxins or by-products of degenerating proglottids, or due to mucosal irritation.

D. latum infection results in hyperchromic, megaloblastic anemia with thrombocytopenia and leukopenia. Anemia seen in diphyllobothriasis is typically similar to

that seen in Vitamin B₁₂ deficiency and could be mistaken for pernicious anemia. Worms located high up in the jejunum compete effectively with the host for the Vitamin B₁₂ in the diet. If worms are pushed further down the intestines, with treatment, anemia is relieved. The vitamin B₁₂ content of *D. latum* is approximately 50 times that of *T. saginata*.

Diagnosis

Residence in or travel to an endemic area, a raw-fish diet, and a pernicious type of anemia may be suggestive of diphyllbothriasis. Definite diagnosis is made on finding the characteristic operculated eggs or on occasion, proglottids in stools. Sometimes, proglottids may be vomited. Since eggs are usually numerous, direct fecal smears usually suffice. The Kato technique is also useful in demonstrating eggs.

To differentiate anemia due to diphyllbothriasis from pernicious anemia, examination of the gastric juice for the presence of free hydrochloric acid is useful. Pernicious anemia is associated with achlorhydria.

Treatment

The drug of choice is praziquantel as 5 to 10 mg/kg single dose. The criterion for cure is recovery of the scolex in feces after treatment. If the scolex is not recovered, a repeat stool examination is done after 3 months to be certain that the patient is no longer infected.

Epidemiology

Human infection is dependent on the presence of human or animal definitive hosts, the presence of suitable intermediate hosts, dietary habits, and amount of pollution of fresh waters. The preference for eating raw fish and the lack of sanitary toilet facilities contribute to the transmission of the parasite. Although other mammalian hosts like dogs, cats, and bears exist as reservoir hosts, man is responsible

for the propagation of the infection in the endemic areas.

D. latum is prevalent in the temperate zones where the population has a habit of eating raw or improperly cooked fish. It is present in the Baltic countries, Switzerland, Romania, and the Danube Basin. In Asia, it can be found in Russia, Turkistan, Israel, Northern Manchuria, and Japan. In the Americas, it can be found in Chile, Argentina, and in some North American states and Canada. Seven human infections have been documented in the Philippines.

Prevention and Control

All freshwater fishes should be thoroughly cooked. Freezing for 24 to 48 hours at a temperature of -18°C kills all plerocercoids. In endemic areas, prevention should center on controlling the source of infection, proper disposal of sewage and marketing of fish.

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Extraintestinal Cestodes

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes

Echinococcus spp.

Human echinococcosis is regarded as an emerging/re-emerging zoonotic disease. The disease is caused by the larval stage of *Echinococcus* spp., which is acquired when the eggs of this parasite are ingested. *Echinococcus* spp. belong to the Family Taeniidae, Order Cyclophyllidea. There are six recognized *Echinococcus* species, four of which are of public health importance. *E. granulosus* and *E. multilocularis* cause cystic echinococcosis and alveolar echinococcosis, respectively, while *E. vogeli* and *E. oligarthrus* both cause polycystic echinococcosis. *E. multilocularis*, *E. vogeli*, and *E. oligarthrus* are less common because their life cycles are sylvatic.

Parasite Biology

The adult worm inhabits the small intestines of canines. It measures 3 to 6 mm in length and possesses a pyriform scolex, a short neck, and three proglottids: one immature, one mature, and one gravid. The scolex is typically taeniid in that it has four acetabula. It is armed with 30 to 36 hooks. The gravid proglottid is usually the widest and the longest proglottid. The uterus is midline, with lateral evaginations, and is filled with eggs which resemble those of other taeniid worms. Eggs may be released inside or outside the host.

The eggs are swallowed by suitable intermediate hosts, such as goats, horses, camels, and sheep. Man may also accidentally ingest the eggs. The eggs hatch in the duodenum and release oncospheres that penetrate the intestinal wall of the intermediate host. The oncospheres then migrate into the mesenteric venules which lead them to various organs and tissues where they eventually lodge and develop into cysts.

The larval stage, called hydatid cyst, is formed through central vesiculation. Cysts may grow at rates ranging from 1 to 5 cm in diameter per year. Numerous protoscolices may be found within the cyst. Development is completed when the cysts in tissues are ingested by carnivores or omnivores. Once inside the definitive host, the protoscolices evaginate and attach to the intestinal wall where they develop into adults. They reside in the small bowel of the host where they start to release eggs that are then passed out in the stool (Figure 4.7).

The hydatid cysts usually measure 1 to 7 cm in diameter. The cyst has an outer laminated hyaline layer and an inner nucleated germinal layer. Protoscolices may be found in brood capsules, which contain only the germinal layer, and daughter cysts which are replicas of the mother cysts. The brood capsule may rupture and release protoscolices. Protoscolices and brood capsules that lie free in the cyst are referred to as hydatid sand (Plate 4.16). Up to 2 million protoscolices may be found in an average cyst.

Pathogenesis and Clinical Manifestations

Pathology of human cystic echinococcosis is caused by the developing larval cyst in the tissues of the intermediate host. The most common and most important site of involvement is the liver, which is seen in 70% of the cases, 85% of which is located in the right lobe. The lungs are involved in 20 to 30% of cases, while other organ involvement, such as the brain and the orbit, make up 10% of cases. Cysts are less commonly seen in the spleen, kidneys, heart, bone, and central nervous system. The cysts of *E. granulosus* are called unilocular hydatid cysts, while those of *E. multilocularis* are considered alveolar cysts. As the unilocular cyst

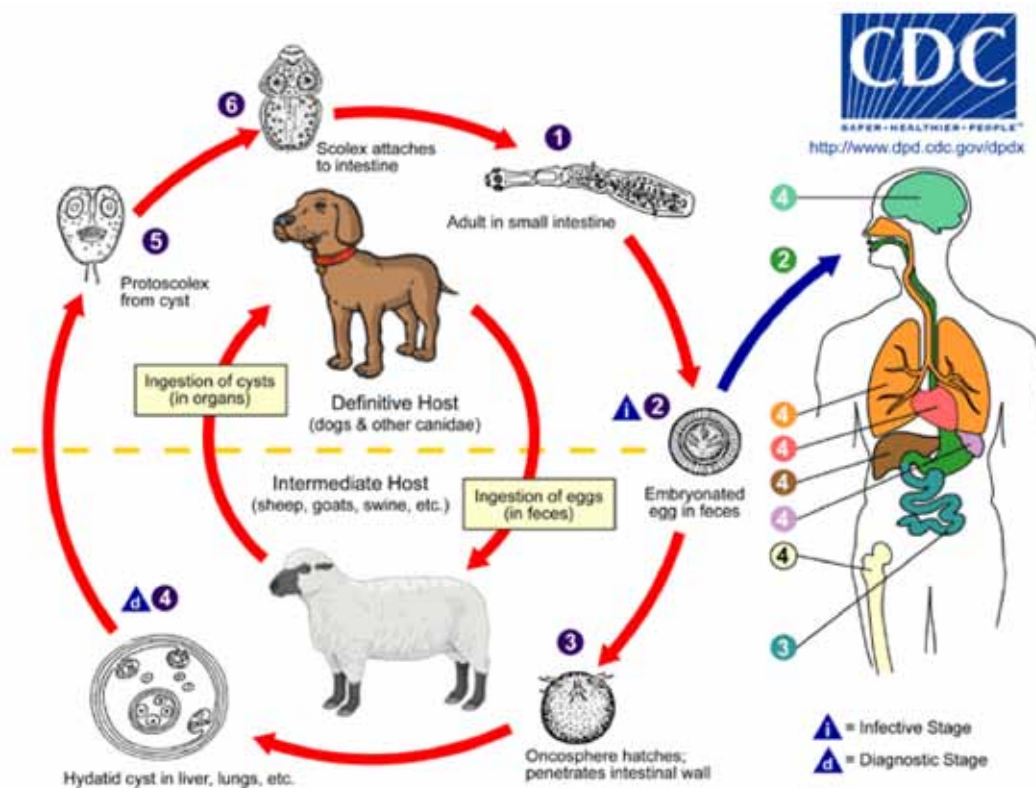


Figure 4.7. Life cycle of *Echinococcus* spp.
 (Accessed from www.dpd.cdc.gov/dpdx)

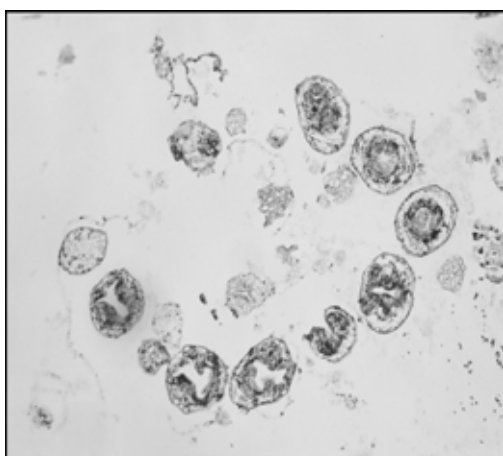


Plate 4.16. Hydatid sand
 (Courtesy of the Department of Parasitology,
 UP-CPH)

develops, inflammatory reactions may occur in surrounding tissues. Recent studies in mice have shown that infection with *E. granulosus* leads to down-regulation of inflammatory cytokines, resulting in local immunosuppression. This may be the mechanism by which the parasite is able to escape host cell-mediated response. Mass effect brought about by the enlarging cyst results in organ impairment as the neighboring tissues undergo atrophy and tissue necrosis.

Although echinococcal infection may be acquired during childhood, infections involving the liver and the lungs are often diagnosed in adults due to the cysts' slow growing nature. Simple or uncomplicated cysts may not produce any symptoms, and patients may harbor the cysts for years. In some cases, the presence of the cyst is only an incidental finding in routine

radiographic examination. Once symptoms start to occur, they typically reflect the site of involvement. Hepatic cysts are mostly found in the inferior right lobe, and may present as hepatic enlargement, right epigastric pain or jaundice. Abdominal cysts may cause discomfort when the cysts are large enough. Cysts may rupture from coughing, muscle strain, trauma, aspiration, and operative procedures. When this happens, the protoscolices, brood capsules, and daughter cysts may metastasize and reach other tissues to develop into secondary cysts after 2 to 8 years. Cysts may also become intrathoracic if they are located in the superior lobe of the liver and rupture into the thoracic region.

The rupture of a hepatic cyst into the biliary duct produces a characteristic triad of findings: intermittent jaundice, fever, and eosinophilia. Peribronchial cysts may discharge into a bronchus and result in sudden coughing accompanied by allergic symptoms. Sputum may contain frothy blood, mucus, hydatid fluid, and bits of membrane. Involvement of the brain may cause increased intracranial pressure and Jacksonian epilepsy. Renal involvement may cause intermittent pain, hematuria, kidney dysfunction, and hydatid material in the urine. Secondary infection of the cyst may also occur. Bacteria may enter the cyst and lead to pyogenic abscess formation. A patient with this condition usually presents with chills and high fever.

Secondary cysts and infected cysts result in higher mortality rates. In cases where primary cysts rupture, serious anaphylaxis may result from a large amount of hydatid material entering the bloodstream. Multiple cysts on different major organs, seen in 20 to 40% of infected individuals, may consequently result in multiorgan failure. Intrabiliary rupture of the cyst is the most common complication, followed by suppuration.

Diagnosis

Radiographic findings and/or ultrasonography, combined with a history

of residence in an endemic area, and close association with dogs are important in the diagnosis of echinococcosis. The World Health Organization (WHO) has developed a standardized classification system for hepatic cysts detected by ultrasonography, as shown in Table 4.1.

Positive serologic tests, such as the use of indirect hemagglutination (IHA), indirect fluorescent antibody (IFA) test, and enzyme immunoassays (EIA) are adjunct to radiologic

Table 4.1. WHO classification for hepatic echinococcal cysts

Classification	Description
Type CL	Unilocular cystic lesion(s) with uniform anechoic content without pathognomonic signs
Type CE1	Unilocular cysts with uniform anechoic content and with pathognomonic signs that include visible cyst wall and 'snow flake sign'
Type CE2	Multivesicular, multiseptated cysts
Type CE3	Anechoic content with detachment of laminated membrane from the cyst wall visible as floating membrane or as 'water-lily sign'
Type CE4	Heterogeneous hypoechoic or hyperechoic degenerative contents, no daughter cysts present
Type CE5	Cysts characterized by thick calcified wall which is arch-shaped, producing a cone-shaped shadow, the degree of calcification may vary from partial to complete

diagnosis. These tests have sensitivities ranging from 60 to 90% and may be used as screening tests. Although serology may be useful to confirm presumptive diagnoses, one must be wary of false positive findings which may occur if the patient is infected with other helminths, or if he has a chronic immune disease. A negative finding, on the other hand, will also not completely rule out the disease since some cyst carriers have undetectable antibodies. Serology may have a relatively high sensitivity (80-100%) and specificity (88-96%) if cysts are located in the liver, but when cysts are located in other

organs such as the lungs and the brain, the serodiagnostic reactivity is lowered, decreasing the reliability of this adjunctive diagnostic test. Detection of IgG antibodies to hydatid cyst fluid-derived native or recombinant antigen B subunit, through ELISA or immunoblot, is the current gold standard serology for human cystic echinococcosis.

Treatment

Surgical resection is still considered the preferred treatment for echinococcosis presenting with a large (>10 cm in diameter) liver cyst, secondary infection, or cysts in extrahepatic sites. Small (<7 mm in diameter), isolated cysts, uncomplicated cysts, and patients with negative serology respond best to chemotherapy with benzimidazole compounds. Treatment with albendazole (10-15 mg/kg/day) or mebendazole (40-50 mg/kg/day) for a minimum of three months has been demonstrated to be effective. Percutaneous aspiration, injection, re-aspiration (PAIR) technique may be indicated for patients with single or multiple cysts in the liver, abdominal cavity, spleen, kidney, or bones, who cannot undergo surgery. This technique involves: (a) ultrasound-guided percutaneous puncture, (b) aspiration of substantial amounts of cystic fluid, (c) injection of a protoscolicidal agent (e.g., 95% ethanol or hypertonic saline) for at least 15 minutes, and (d) re-aspiration. Treatment with PAIR plus albendazole or mebendazole has been shown to have greater efficacy and lower rates of morbidity, mortality, and disease recurrence.

Epidemiology

Cystic echinococcosis is the most common presentation of echinococcal infection in humans, accounting for >95% of global cases, with a burden of disease of about one million disability-adjusted life years (DALYs). There are approximately 2 to 3 million cases of human cystic echinococcosis, and 0.3 to 0.5 million cases of human alveolar echinococcosis

worldwide. Cystic echinococcosis is most prevalent in countries in the temperate zones, such as southern South America, the Mediterranean, southern and central parts of Russia, Central Asia, China, Australia, and parts of Africa. Reemergence of cases have been reported in Bulgaria, where the incidence of echinococcosis in children increased from 0.7 to 5.4/100,000 between the 1970s and the mid-1990s. Similarly, prevalence of infected canines in Wales doubled between 1993 (3.4%) and 2002 (8.1%).

Filipinos who have traveled to or worked in endemic areas may get infected. A 35-year old Filipino overseas contract worker in the Middle East presented with a right hilar mass on routine chest x-ray. Thoracotomy showed a 10 cm by 6 cm cystic mass containing hydatid sand. Another Filipino overseas contract worker from Iraq presented with a growing mass in the hip area. Biopsy results showed presence of hydatid sand. More recently, a Filipino female, with no apparent history of travel to an endemic area, consulted her physician for neurologic symptoms. Histopathologic findings of tissue obtained during neurosurgery also showed the presence of hydatid sand.

Prevention and Control

Prevention is achieved by reducing the infected populations and by minimizing opportunities for transmission. Regular testing and quarantine, and treatment of dogs with praziquantel in endemic areas are important control strategies that have resulted in the reduction of echinococcosis cases. To minimize transmission, dogs should not be allowed in slaughterhouses, and refuse from these facilities should be sterilized or properly disposed. Health education should include knowledge on the mode of transmission, and should emphasize the danger of intimate contact with dogs. New strategies for the control and prevention of echinococcosis include vaccination of livestock, which has been proven to provide

>95% protection against *E. granulosus*, as well as the development of more sensitive diagnostic techniques for definitive and human hosts.

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Spirometra spp.

Winifreda U. de Leon

Sparganosis refers to the larval infection with the plerocercoid larvae, also known as spargana, of pseudophyllidean tapeworms falling under the Genus *Spirometra*. There are many species of *Spirometra*, but those commonly involved in human sparganosis are *Spirometra mansoni*, *Spirometra erinacei*, and *Spirometra ranarum*. Adults of these worms are intestinal parasites of cats, dogs, and other carnivores.

Parasite Biology

The gravid proglottids of *Spirometra* sp. have a spiral uterus, in contrast to the rosette uterus observed in *Diphyllobothrium* sp. *Spirometra* eggs are operculated and immature, similar to those of *Diphyllobothrium*, although smaller.

Spirometra eggs are passed out with the feces of the definitive hosts and become embryonated in water (Figure 4.8). The coracidium, once

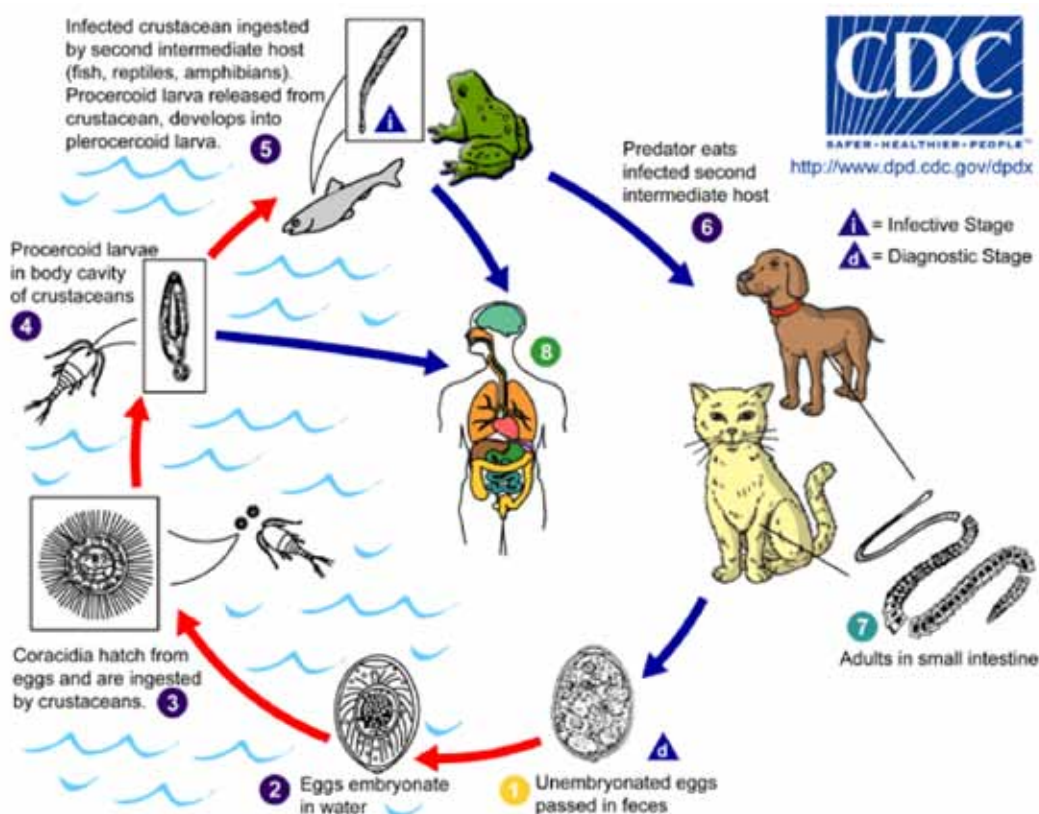


Figure 4.8. Life cycle of *Spirometra* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

released, infects *Cyclops* and develops into the procercoid larva. Once the infected *Cyclops* are ingested by the secondary intermediate hosts such as frogs, snakes, and chickens, the procercoid larva develops into the plerocercoid larva which is also known as sparganum (pl. spargana). If the infected tissues of the second intermediate host are ingested by the definitive host (cats and dogs), the plerocercoid larva develops into an adult worm. These adults are usually mistaken for adult *Diphyllbothrium latum*, although *Spirometra* sp. adults are shorter.

Pathogenesis and Clinical Manifestations

Humans may be infected through: (a) drinking water containing *Cyclops* or copepods infected with procercoid larvae; (b) eating infected second intermediate hosts like frogs, toads, or snakes containing the plerocercoid larvae; (c) applying plerocercoid infected flesh of frogs and snakes as poultices on sores on the eye, vagina, and skin resulting in subsequent penetration into cutaneous tissues; and (d) consumption of infected flesh of paratenic hosts like wild pigs. The resulting condition is called sparganosis.

The larvae may be found in any part of the body. Most commonly, they are found in and about the eyes, in the subcutaneous and muscular tissues of the thorax, abdomen, thighs, inguinal region, and in the viscera. Patients may complain of painful edema due to migrating larvae, hence, the condition is also known as migrating tumor. Local indurations, periodic giant urticaria, edema, and erythema with chills, fever, and high eosinophilia may be seen in patients.

Diagnosis

Sparganosis is diagnosed through the recovery of the plerocercoid larvae from infected tissues. The larvae that are opaque and glistening white usually measure about 3.5

cm in length. When the larvae are flattened, a spatulate scolex can be appreciated, together with pseudosegmentation, and a slit like invagination at the anterior end. Species identification, however, can only be done through experimental animal infection.

Treatment

The main form of treatment is surgical removal of the larvae from the infected tissues. Praziquantel has been recommended, but its efficacy in humans has not been proven.

Epidemiology

Cases of sparganosis have been reported worldwide: in Africa, India, Holland, Australia, and South America. In Asia, the majority of cases came from Japan, Korea, Thailand, Malaysia, and Indonesia.

The first case reported in the Philippines was in 1935, when a sparganum was recovered from the abdominal wall of a seminarian originally from Pulilan, Bulacan. The second case, reported in 1950, was that of a fisherman from Libon, Albay, presenting with a 4 cm lower chest lump. The third case, reported in 1953, was that of a 50-year old nun, also from Pulilan, Bulacan, complaining of an erythematous, slightly painful, pruritic mass in the inner aspect of the thigh. Although two of the cases were from the same place, it was possible that the nun got infected during her stay as a missionary in Mindanao. A fourth case, reported in 1962, was that of a 46-year old female with a slightly painful, subcutaneous nodule at the base of the neck. In the late 1970's, and the early 1980's, two more cases of human sparganosis were confirmed at the Department of Parasitology, College of Public Health, University of the Philippines Manila.

In all six cases, the spargana were motile upon excision of the mass. With the last two cases, the spargana showed the typical solid body with worm-like appearance. There

was pseudosegmentation with a slit-like invagination at the head end. None of the patients gave a history of consuming raw frogs, birds, or snakes, nor did they admit having applied the flesh of such animals as a poultice. Presumably, transmission may have occurred through drinking water with *Cyclops* containing procercoids.

To date, there have been other cases of human sparganosis encountered in the Philippines. One interesting case was the involvement of the central nervous system of an adult female Filipino complaining of headache, seizures, confusion, and hemiparesis. On computed tomography scan, an area of low density, distinct from other brain lesions, was detected. Multi-Dot ELISA technique on the serum and the cerebrospinal fluid of the patient was positive for *Spirometra* antigen, but not for cysticercus or *Paragonimus* antigens. The positive reaction was confirmed using the MicroPlate ELISA procedure. The infection may have been acquired through drinking of water contaminated with infected *Cyclops*.

Infection can be prevented by drinking boiled or filtered water, by cooking possible intermediate and paratenic hosts thoroughly, and by avoiding the practice of applying the flesh of frogs to inflamed areas.

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CHAPTER 5

Trematode Infections

Blood Flukes

Edsel Maurice T. Salvaña, Vicente Y. Belizario, Jr.

Schistosoma is a genus of parasitic blood flukes that infect birds and mammals, including humans. Five species of medically important *Schistosoma* have been identified: *S. japonicum*, *S. mansoni*, *S. haematobium*, *S. mekongi*, and *S. intercalatum*. *S. japonicum* is the predominant species in the Philippines and will be discussed in detail in this chapter.

Schistosoma japonicum or the Oriental blood fluke causes schistosomiasis japonica. It is endemic in China, the Philippines, and Indonesia. It was first described in Japan but has been eliminated, with the last human case reported in 1977. For centuries, schistosomiasis has caused significant morbidity and mortality. *S. japonicum* eggs have been identified in a female corpse from the Western Han Dynasty, 2,000 years ago. While the disease was described as early as 1847 by Fuji, the adult *S. japonicum* was first described by Katsurada only in 1904.

The first Chinese case was diagnosed by Logan in 1905, and Wooley reported the first case in the Philippines in 1906. Strains of *S. japonicum* from the different geographic regions are genetically distinct but all require snails of the species *Oncomelania* as intermediate hosts. Phenotypic variations include minor morphological characteristics, infectivity to *Oncomelania* snails from different areas, periodicity of cercarial emergence, ability to develop in different definitive hosts, growth rates, egg production, pre-patency periods,

pathogenicity, and immunogenicity. Injection of irradiated cercariae of the Chinese strain confers resistance against the homologous strain but not against the Philippine strain. The mouse pathogenicity of the Chinese strain is less than that of the Philippine strain.

Differences also seem to exist among the various island strains (Mindoro, Leyte, Sorsogon, and Mindanao) in the Philippines. However, no studies have definitively showed variations and similarities in host range, pathogenicity, susceptibility to chemotherapeutic agents, and other characteristics among these strains. Most studies on different aspects of the biology of *S. japonicum* have been done on the Leyte strain, with the findings extrapolated for other island strains.

Parasite Biology

The *S. japonicum* life cycle involves an intermediate snail host and a definitive mammalian host, with free-living stages in between (Figure 5.1). Embryonated eggs from the stool of a definitive host come into contact with fresh water and hatch within 2 to 4 hours into free-swimming miracidia. Miracidia seek out and infect the snail intermediate host, *Oncomelania hupensis quadrasi*, and develop into sporocysts. Sporocysts are able to reproduce asexually and can later give rise to free-swimming cercariae after 60 to 70 days. The cercariae penetrate the skin of the definitive

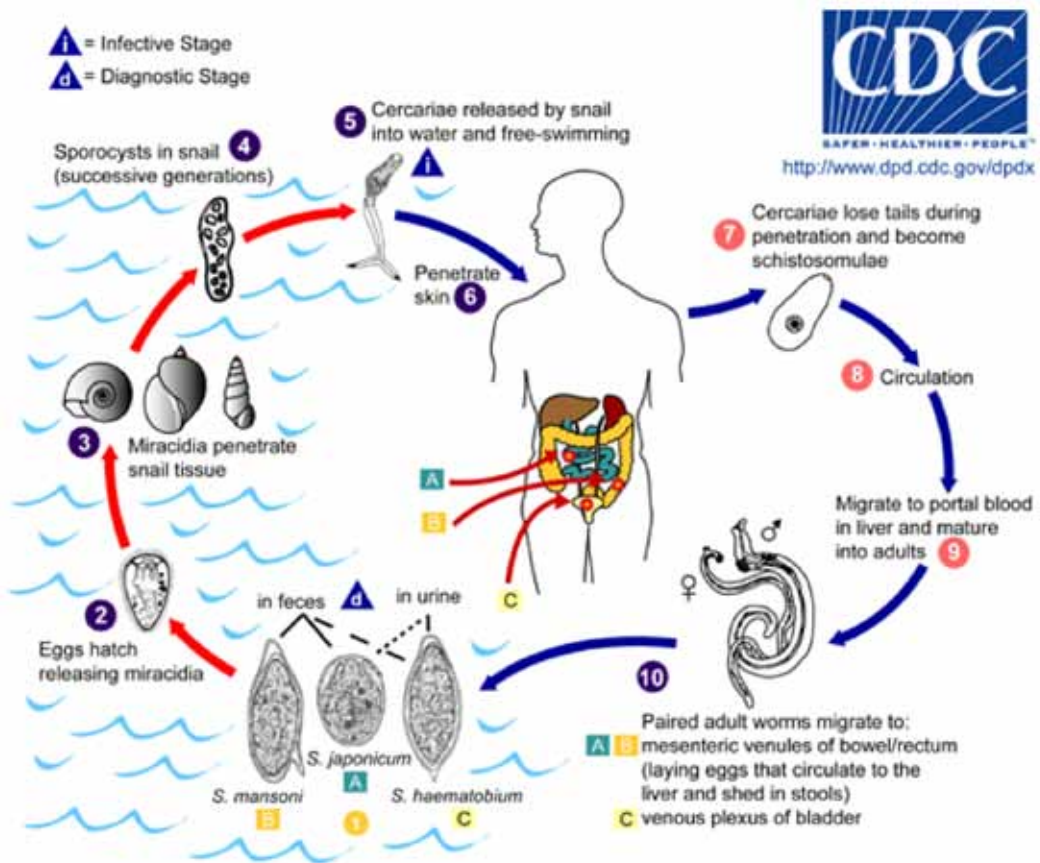


Figure 5.1. Life cycle of *Schistosoma* spp.
 (Accessed from www.dpd.cdc.gov/dpdx)

host when the host comes into contact with infested fresh water. Cercariae then lose their tails and transform into schistosomula and enter superficial lymphatic vessels or subcutaneous veins and reach the lungs. Most authors believe that from the pulmonary circulation, the schistosomules migrate intravascularly to reach the portal vein where they mature. However, there is some evidence that schistosomules can escape from the lungs into the pleural cavity and pass through the diaphragm into the liver to reach the portal vein. In the portal circulation, schistosomules differentiate into male and female forms and pair up, with the larger female occupying the gynecophoric canal on the adult male (Plate 5.1). Each female fluke deposits 500

to 2,000 immature eggs/day in the branches of the portal vein. These require 10 to 12 days to mature and embryonate. Eggs deposited



Plate 5.1. *Schistosoma japonicum* male (left) and female (right) (Courtesy of the Department of Parasitology, UP-CPH)

in mucosal or sub-mucosal terminal veins or capillaries escape through ulcerations into the intestinal lumen and are subsequently exported with the feces. Egg deposition usually begins from the 24th to the 27th day after cercarial penetration.

While the intermediate snail host is specific for each schistosome species, *S. japonicum* has a wide range of definitive hosts including domestic mammals such as dogs, pigs, cats, carabaos, and cows, along with sylvan reservoirs such as rodents and monkeys. Susceptibility to infection can vary among different definitive hosts. Some hosts are considered permissive, i.e., *S. japonicum* matures and oviposits over an extended period (e.g., humans, monkeys, rabbits, and mice); while others are non-permissive wherein schistosomes are stunted or they may mature but die out prematurely.

Infection rates can also vary between individuals of the same species. This is likely due to variations in immune activation and response, and has been demonstrated in different genotypes (e.g., inbred strains of mice). Some evidence suggests that in a particular endemic island of the Philippines, only one strain is common to the different definitive hosts. A large series of experimental crosses of cercariae originating from a single miracidium obtained from different naturally infected mammalian hosts from Leyte was made between 1954 and 1957. All of the crosses of flukes of different vertebrate origin were successful. It is easy to presume that these crossings occur in the transmission sites in nature and that only one strain of *S. japonicum* exists in this endemic island.

S. japonicum egg is ovoid, round or pear-shaped, and is pale yellow in color. The longer diameter ranges from 46 to 110 μm , while the shorter diameter ranges from 37 to 90 μm . It has a thin shell onto which residual tissue or red cells may be adherent. A curved hook or spine may be observed near one of the polar ends, but only if the egg is properly oriented (Plate

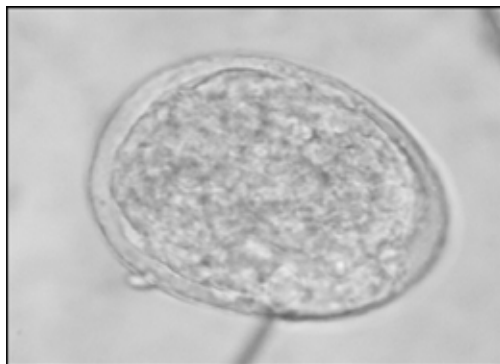


Plate 5.2. *Schistosoma japonicum* egg
(Courtesy of the Department of Parasitology,
UP-CPH)

5.2). Eggs are in the multicellular stage when released from the adult female and require 10 to 12 days to embryonate and mature. Immature eggs passed out with feces no longer mature in the soil and are not viable. Mature eggs in feces can survive and still hatch for up to a week if desiccation is slow. In view of the nature of the rainfall distribution in endemic areas of the Philippines, the prolonged survival time of the ovum increases the probability that the eggs will be washed down to a water course where snails are present.

Eggs hatch only in clean fresh water with sufficient oxygen. They will not hatch in salinity greater than 0.7% or at mammalian body temperatures. A temperature of between 25 to 31°C in slightly alkaline water is ideal. Hatching occurs almost instantaneously upon immersion in water. Most viable eggs will hatch within 2 to 4 hours. Many miracidia can survive overnight. Essential morphological features of the miracidium include an apical papilla, epidermal plates covered with cilia, a primitive gut, a pair of cephalic unicellular penetration glands opening by a duct at the base of the apical papilla, two pairs of flame cells, and germinal cells. The miracidia are phototactic and swim actively in surface water. They remain infective for snails for 8 to 12 hours, but infectivity diminishes with time.

The mechanism by which snail intermediate hosts are located and infected by miracidia, as well as what may divert them from infecting snails has not been well-elucidated. Although it is postulated that secretions or excretions of *O. h. quadrasi* attract the miracidia, but these chemotactic molecules have not yet been identified. In early experiments performed in Leyte, initial contact between a single miracidium placed equidistant from *O. h. quadrasi* and other snails was purely random.

After contact with soft parts of the snail, miracidial penetration is effected by movement and the lytic action of cephalic gland secretions. Factors that influence the infection of snails by miracidia include the age of the snails and the miracidia, the number of miracidia per snail, the length of contact time, water flow, and turbulence.

The ciliated surface of a miracidium disappears once penetration is completed. Within several days, the miracidium develops into a first generation or mother sporocyst near the point of entry. At 96 hours after penetration, it transforms into an elongated sac filled with germinal cells. On the 8th day, germ cells bud off the epithelial lining and develop into daughter sporocysts. These migrate through loose connective tissue to the liver. In the connective tissues of the liver, further development of germ balls into daughter sporocysts takes place. Free swimming cercariae are ultimately produced.

Thus, from a single miracidium, through the process of asexual multiplication within the mother and daughter sporocysts, scores of cercariae of a single sex are produced. The limiting factor for the number of cercariae that develop from one miracidium is the size of the snail host. In *S. mansoni* and *S. haematobium*, thousands of cercariae are produced since their snail hosts are much larger.

Only a relatively small proportion of the miracidia that enter snails eventually produce cercariae. Only 6 to 10% of exposed *O. h. quadrasi* found in a study done in Mindoro

shed cercariae. Mortality among infected snails is increased in comparison with uninfected snails. Infected *Oncomelania* have decreased egg-laying capacity.

Mature cercariae emerge from daughter sporocysts and escape from the snail into fresh water. The cercaria has a body and a forked tail. The main body measures from 100 to 500 μm in length and 40 to 60 μm transversely. The tail trunk is 140 to 150 μm by 20 to 35 μm ; and the fork is 50 to 70 μm long. The cercaria has an oral sucker, which occupies the anterior end of the body, and a small ventral sucker. Cercarial penetration is mediated by lytic enzymes secreted by cephalic glands and aided by muscular activity.

There are several ways by which cercariae emerge from snails infected by miracidia. Singly infected snails may shed cercariae as early as the 42nd day after miracidial penetration, although the average time is 62 days. Multiply infected snails take somewhat longer, but shed more cercariae and have a longer shedding period. The total number of cercariae shed during the whole length of infection is about 230 for singly infected snails and 280 for snails with multiple infections. On the average, a snail sheds only about two cercariae per day. Snails may climb vegetation above the water line or get stranded on the dryer portion of the snail habitat for several days. Because *O. h. quadrasi* can easily withstand drying for 7 to 10 days because of its operculum, it may shed scores of cercariae upon re-entry into water. This phenomenon is exploited in the laboratory to recover more cercariae. Snails are taken out of the aquaterraria for 2 to 4 days before these are crushed or made to shed the parasites.

Studies done in Leyte indicate that cercariae are most abundant in the field during the early evening hours. These observations parallel those of Bauman et al. who also found that the natural release of cercariae is nocturnal, occurring from early evening to midnight. Two factors have been proposed to explain this occurrence: the

negative effects of exposure to sunlight, and the fact that *O. b. quadrasi* is more active and mobile at night, allowing it to reach water sources more often in the evening. Cercariae can survive for up to 24 hours after release, and so infested water can be infectious at any time during the day.

Cercariae swim on the surface of the water, which facilitates contact and attachment to the skin of the host. Host identification by *S. japonicum* seems to be non-specific, although in thermal gradients they show a preference to a temperature of $35^{\circ}\text{C} \pm 3^{\circ}\text{C}$. Penetration is stimulated by skin lipids. Some chemicals like dimethylate and niclosamide repel cercariae when applied to the skin. However, routine use is impractical due to the need for frequent reapplication.

After skin penetration, the cercaria loses its tail and transforms into a schistosomule. Schistosomules have adapted to survive in serum or physiologic saline at 37°C . The cercarial tegument is replaced by a five to seven-layered membrane. In the laboratory, a cercaria can be transformed into a schistosomule by repeated passage through small bore syringe needles resulting in shearing off of the tail, by passage through isolated skin, and by application to a surface with skin lipids or crude egg lecithin. One study showed that schistosomules can be found in the pleural cavity on the 2nd day of infection, in the parenchyma of the diaphragm on the 4th day, in the liver parenchyma on the 6th day, and in the intrahepatic branches of the portal vein afterwards. There was a very close correlation between the number of superficial lung petechiae on the 4th to 6th day after cercarial penetration, and the number of flukes recovered at day 30 from the portal system by perfusion. These observations seem to indicate that schistosomules break out of the pulmonary microvasculature and traverse the lungs to escape into the pleural cavity. They later go through the diaphragm, enter the peritoneal space and penetrate the liver to reach the

intrahepatic portions of the portal vein. This is likely the more common path of migration to the portal circulation, while the vascular route via the arterial circulation may be a secondary pathway.

Unlike other trematodes, schistosomes are dioecious. Adults have a large sucker capping the anterior end, a ventral sucker, and a gonophore, located slightly posterior to the ventral sucker. The suckers aid in movement and enable the flukes to maintain their position inside veins. The male is the shorter but sturdier sex and measures 12 to 20 mm in length by 0.4 to 0.5 mm in diameter. It has a gynecophoral canal where the longer and more slender female is held (Plate 5.3). Females measure 15 to 26 mm by about 0.3 mm. They can live for up to 30 years but the mean life span is much shorter (3-8 years). In the male, the testes are arranged in one row above the ventral sucker, while in the female, a single pyramidal ovary is located in the midline.

Schistosomes have an incomplete digestive system and an excretory system made up of flame cells. These internal structures are surrounded by circular and longitudinal muscles. The worms ingest red blood cells and possess a protease (hemoglobinase) that breaks down globulin and hemoglobin. They



Plate 5.3. *Schistosoma japonicum* adults in copula (Courtesy of the Department of Parasitology, UP-CPH)

utilize glucose at a rapid rate and likely absorb nutrients through the body wall. More research is needed to elucidate the mechanisms for nutrient uptake and metabolism including enzyme systems.

Pathogenesis and Clinical Manifestations

Cercarial penetration of skin is usually accompanied by dermatitis with pruritus and localized reaction known as “swimmer’s itch.” This is similar to that seen from non-japonicum and non-schistosome cercariae that do not lead to chronic disease in humans. The manifestation is self-limited and repeated cercarial exposure causes these acute reactions to wane over time. Non-endemic travelers to endemic areas are the most likely to experience this phenomenon.

Typically after 2 to 12 weeks following cercarial penetration, schistosomule migration can give rise to a syndrome characterized by easy fatigability, respiratory symptoms, arthralgias, myalgias, malaise, eosinophilia, fever, and abdominal pain, which has been termed “snail fever,” Katayama fever, or Katayama syndrome. The latter term is currently preferred since not all patients may present with fever. Hepatosplenomegaly is not uncommon and can be quite debilitating during this period of infection, and in rare cases may lead to severe hepatic dysfunction and death. Migration through the pulmonary circulation can cause wheezing and coughing. Aberrant migration of maturing schistosomules may occlude the circulation of the brain and the spinal cord precipitating seizures, paresthesias, transient ischemic attacks, and strokes. While most patients will get better without medication, treatment with anthelmintics usually leads to faster resolution of symptoms.

The main pathology and chronic disease manifestations of schistosomiasis japonica are due to the host granulomatous reaction to eggs deposited in the liver and other organs. Since *S. japonicum* does not multiply in the definitive host, the initial quantum of cercariae that infect

the host and mature to lay eggs determine the severity of infection, with repeated infection from continuing exposure causing the most severe burden of disease. Correlations between excretal egg-output, number of resident flukes, and egg counts in the liver have been shown in experimentally infected monkeys.

Egg deposition can occur in any organ, but those most commonly involved are the liver, intestines, lungs, and much less frequently, the central nervous system. In whatever organ the eggs are entrapped, the primary lesion is a granulomatous hypersensitivity reaction around a single egg or egg cluster. Since *S. japonicum* typically deposits eggs in clusters, very large and destructive granulomas are formed. After initial egg deposition, there is an accelerated formation of larger and more destructive granulomas. However, as the infection becomes chronic, granulomas become smaller or modulated. Appreciation of the immunologic basis of this phenomenon raises the question of vaccination against the disease. Immunization to promote a modulated granuloma response could lead to a reduced likelihood of developing severe hepatosplenic disease.

In view of the collateral circulation established, eggs are shunted into the systemic circulation and filtered in the pulmonary microvasculature, eventually causing pulmonary hypertension.

The clinical course of infection is arbitrarily divided into three stages, namely: (a) incubation (corresponding to the period from cercarial penetration and schistosomular migration to the time the flukes mature); (b) period of early egg deposition and extrusion; and (c) period of tissue proliferation. Since there is a significant overlap of the second and third stages of the disease due to repeated infection, it is usually more useful to refer to organ involvement as the basis for clinical classification or description.

American soldiers who landed in Leyte in 1944 and acquired schistosomiasis became subjects for the study of early manifestations.

Among 42 soldiers studied, itching soon after exposure was noted in four cases. In another series of 41 patients, only one experienced itching. The majority of subjects had chills, fever or non-productive cough during the period corresponding to larval or schistosomular migration. Another longitudinal study involving 337 cases established that the pre-patent period ranged from 42 to 52 days.

Colonic involvement in schistosomiasis japonica starts during the early period of egg deposition. Ulcerations caused by eggs result in dysentery or diarrhea, depending on the worm burden. In the chronic stage, colonic schistosomiasis is usually asymptomatic, although there may be occasional bouts of diarrhea. Chronic colonic schistosomiasis has been observed as an incidental finding with some malignancies, but a causal relationship has not been established.

Hepatosplenic disease is the most serious consequence of chronic schistosomiasis. It is characterized by hepatosplenomegaly, portal hypertension, ascites, and development of collateral circulation, which can lead to esophageal and gastric varices. An analysis by Pesigan et al. of 2,540 cases of schistosomiasis japonica detected by stool examination during surveys of Department of Health teams in 1950 and 1951 showed that 31% had developed mild hepatosplenic disease, 9.1% had definite signs of ascites, and 1.4% had severe portal hypertension with prominent ascites (Plate 5.4). Cinco et al. reported that 14% of cases of schistosomiasis had a history of hematemesis and/or melena.

Pulmonary involvement may initially occur during the period of larval migration, which can result in coughing, wheezing, and other respiratory symptoms. In chronic schistosomiasis, the lungs follow the liver and intestines in having the most number of schistosomal lesions. Cor pulmonale can result from obstruction of the pulmonary vasculature due to granuloma formation and fibrosis. Eggs likely reach the pulmonary circulation via the



Plate 5.4. A boy from Leyte with portal hypertension and ascites secondary to schistosomiasis (Courtesy of Dr. Edito Garcia)

porto-systemic collateral circulation. Jongco and Flaminiano reported in 1961 that pulmonary schistosomiasis is the most common cause of cor pulmonale in Filipino children. Cor pulmonale may become symptomatic before portal hypertension is clinically apparent and may lead to a delay in diagnosis of schistosomiasis.

Cerebral schistosomiasis (Plate 5.5) is estimated to occur in 1.7 to 4.3% of infections. Among the Americans that landed in Leyte in 1945, 2% had cerebral manifestations. Cerebral manifestations may present as motor or sensory disturbances depending on the site of egg deposition and granuloma formation.

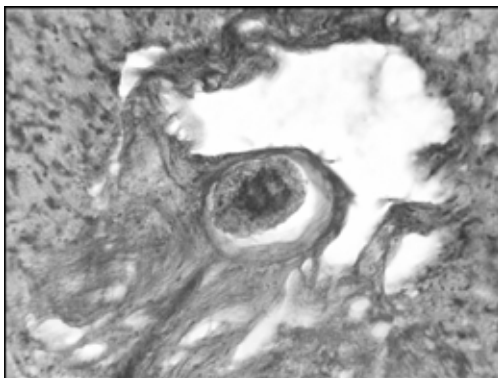


Plate 5.5. *Schistosoma* egg in the brain (Courtesy of the Department of Parasitology, UP-CPH)

Early neurologic involvement is brought about by the parasite's transition from the portal vein via mesenteric and pelvic veins to the spinal veins. Acute cases usually present with fulminating meningoencephalitis with fever, headache, confusion, lethargy, and coma, while chronic cases give a clinical picture of a tumor with localizing signs and increased intracranial pressure.

Diagnosis

Because *S. japonicum* is primarily a parasite of the portal vein and its branches, eggs are not immediately demonstrable in the feces unless they are deposited in the terminal vein or capillaries of the intestinal mucosa or submucosa, and subsequently escape to the intestinal lumen. In infections where there is scarring or fibrosis of sites of ulcerations, passage of eggs into the intestinal lumen can be impeded. In these cases, stool examinations can give negative results even in active infection. Schistosome eggs can also be recovered by rectal or liver biopsy. However, these procedures require specialized equipment and are not practical for mass screening or field surveys. Moreover, tissue diagnosis cannot reliably distinguish active from treated infection.

Microscopic examination techniques are the most specific since these directly visualize the parasite egg. Microscopic techniques include stool examination and rectal imprint. *S. japonicum* eggs tend to clump together, so a small stool sample may turn out falsely negative. This may also occur in cases of light infection.

To establishing a diagnosis, the merthiolate-iodine-formalin concentration technique (MIFC) has sufficient sensitivity for moderate and heavy infections, but it is not adequate for very light infections (<10 eggs per gram of feces). This technique has certain advantages over other stool concentration techniques making it suitable for field surveys. Fecal samples mixed with merthiolate-formalin (MF) solution in screw-capped vials can be

kept indefinitely. Processing can therefore be resumed in the laboratory or at some later convenient time. Protozoans are also preserved and stained in the preparation allowing diagnosis of polyparasitism.

The Kato-Katz technique is the preferred egg-counting technique and is considered the most suitable for quantification of eggs. It is the most commonly used stool examination technique for evaluating epidemiology, effect of control measures, and drug trials. The Kato-Katz preparation can be kept for at least 2 weeks for later examination depending on the workload. There is practically no loss of eggs during storage and processing which makes the technique satisfactory for determining fecal egg density. Specimens with less than 20 eggs per gram of feces require examination of at least three Kato-Katz preparations to have 92% sensitivity.

Rectal snips and imprints require specialized equipment and personnel, but are among the most sensitive techniques. It is also the most invasive since biopsy specimens are required. Another drawback is the inability to distinguish between untreated and treated infection since eggs can persist in rectal tissue long after active infection has been eradicated. Some techniques such as vital staining and egg morphology and embryo motility have been proposed to distinguish viable from nonviable eggs, but none of these are consistently reliable.

The intimate tissue contact between parasite and host during cercarial penetration, schistosomular migration, intravascular growth and development of adults, and deposition of eggs in the tissues stimulate and provoke specific immune responses which can be demonstrated as evidence of infection.

Locally evaluated immunodiagnostic tests include the following: (a) intradermal test for immediate cutaneous hypersensitivity using adult worm extracts; (b) indirect hemagglutination using adult worm and egg antigens; (c) circumoval precipitin test (COPT);

and (d) the enzyme-linked immunosorbent assay or ELISA using soluble antigens of adults and eggs. A multicenter evaluation of *S. japonicum* diagnostics conducted by the World Health Organization, in which the Philippines participated, showed that crude egg antigens were most specific. In view of this, only the COPT, ELISA, and indirect hemagglutination using egg antigens are recommended for use.

There are inherent problems with parasitological diagnosis especially in low endemic areas. Thus, there may be a role for antibody or antigen detection that may have advantages over parasitological diagnosis. In the Philippines, a proportion of COPT positive but single Kato-Katz negatives were shown to have eggs on repeated sampling.

The intradermal test is highly sensitive but nonspecific for infection. It cannot reliably distinguish active from old infection. It is no longer used routinely as other immunodiagnostic tests have replaced it.

Indirect hemagglutination has been shown to be highly sensitive. However, it does require specialized reagents and training but can be performed with minimal equipment in the field. ELISA formats are among the most sensitive tests but the need for laboratory equipment and trained personnel limits its use to banked specimens and cannot be a point of care test. New lateral flow assays, which use card tests with visually apparent results, harness ELISA technology for point of care and field use and have been validated extensively in China. Lateral flow assays are likely the best suited for elimination programs. However, the biggest drawback for antibody detection tests remains the persistence of antibodies long after active infection has been treated.

Antigen detection reflects active infection. In more recent studies, adult worm antigens were found to be better than egg antigens for detecting low level infections (<100 eggs/g). Egg antigens detected only 65 to 85% of cases (n=7), while adult worm antigens detected

55 to 91% (n=14). Urine and serum tests on other schistosome species in Africa and Brazil have so far shown disappointing sensitivity and specificity for antigen based tests. A variety of the currently available antibody and antigen tests should be compared using sera collected from low endemic areas. This will determine which assays are practicable for field use in endemic areas. The method of choice will depend on cost, simplicity, and sensitivity.

The COPT demonstrates the formation of bleb-like or septate precipitates attached to one or more points on the egg surface after incubation of schistosome eggs in a patient's serum. It is currently regarded as the method of choice for definitive diagnosis of this infection in the Philippines. The sensitivity of COPT is due to the fact that it is a microprecipitation reaction visualized under the microscope with sensitivity comparable to passive or indirect hemagglutination.

The COPT may take more than two years to become negative. The time spent in examining is very much reduced with standardized egg preparation obtained from 50 to 60-day old *S. japonicum* infections of rabbits. During this period of infection, there is a maximum proportion of mature eggs from the liver, which can be used as antigens for the test. At least 25% of the eggs can be visualized with precipitates after incubation with a positive serum, so examination of the slide requires a minimum amount of time.

Epidemiological studies in Barrio San Antonio in the town of Basey, Samar, where the whole population was examined using MIFC and COPT, indicated that many infections, particularly of the population above 10 years old were not detected by a single stool examination. Seventy percent of the population tested positive by COPT, while only 40% tested positive with a single stool examination.

Because COPT is technically demanding and requires specialized equipment, it is not routinely used for field testing. Moreover, it

cannot distinguish active from past infection. Currently, COPT is used as an adjunct tool for diagnosis in patients who are stool negative but remain highly suspicious for schistosomiasis. It is not recommended for use as a screening tool in the Philippines.

Treatment

Praziquantel, a heterocyclic prazinoisoquinoline compound, represents a major breakthrough in the treatment of schistosomiasis. It is safe and highly effective in single or divided doses against all the major species of schistosomes. The active substance is a hygroscopic, colorless, almost odorless, crystalline powder with a bitter taste, which is stable under normal conditions but melts and decomposes at 136 to 140°C. It is very soluble in chloroform and dimethyl-sulfoxide, sparingly soluble in ethanol and very slightly soluble in water. Praziquantel is active against adult schistosomes both in vitro and in vivo. In vitro experiments have shown that schistosomes instantly become immobile and undergo contraction on contact with the drug.

Acute toxicity studies conducted in rats, mice, and rabbits have shown that in comparison with other anti-schistosomal drugs, praziquantel has a very low acute toxicity profile. Rats tolerated daily doses of up to 1 mg/kg for 4 weeks, and dogs tolerated daily dosages of up to 180 mg/kg for 13 weeks without organ damage. No effects were seen on the whole reproductive process in rats. Teratogenic effects were not observed in mice, rats or rabbits.

A single dose of 40 to 50 mg/kg, or 25 mg/kg in two doses or three doses of 20 mg/kg given every 4 hours or even a dose as low as 10 mg/kg given three times a day for 2 days provide high cure rates. A dosage of 30 mg/kg given after breakfast and repeated after lunch has been used in trials involving more than 6,000 patients with light to moderate *S. japonicum* infections with a cure rate of almost 90%.

Generally, a single large dose has the same efficacy as several smaller doses at

intervals of several hours. Even if the patient is not fully cured, the passage of eggs becomes significantly reduced. Improvement after treatment is clinically apparent. There is a reduction in the degree of portal hypertension, hepatosplenomegaly, and cerebrospinal manifestations. In local studies, egg reduction rates have ranged from 80 to 96% in patients who received treatment with praziquantel 60 mg/kg in two divided doses.

The frequency of side effects varies in the different treated groups but these are generally mild and transitory. In a retrospective study of 25,693 *S. japonicum* patients treated with praziquantel in China, only 0.4% of patients were reported to have serious adverse effects. In local studies involving more than 6,000 patients, praziquantel given at 60 mg/kg in two divided doses resulted in mild to moderate side effects in 68% while severe reactions were recorded in 1.2%. The most frequent adverse effects are epigastric or diffuse abdominal pain or discomfort, nausea, anorexia, dizziness, headache, and fever. Most of these were noted to be mild and transient.

Artemisinins including artemether have recently been shown to be effective in decreasing *S. japonicum* infections when used as pre-exposure prophylaxis during the planting season in China. Artemether is effective against the juvenile stages of the worm and so this drug is ideal for the non-endemic traveler. However, routine use for endemic natives may be problematic in areas where malaria is co-endemic since this may give rise to resistance. Combination therapy with praziquantel has shown high cure rates in laboratory animals and may be an option in areas with high worm burden or emerging drug resistance.

Epidemiology

Transmission dynamics vary considerably in the different endemic areas due to the many factors that influence the common environment, the behavioral patterns of the definitive host, and the bionomics of the

snail host. Extrapolation of data, whether in snail populations, animal populations, or socioeconomic activities, may not completely capture the true situation. Understanding the epidemiology of schistosomiasis requires the study of the effects of rainfall, socioeconomic activity, cultural and behavioral patterns, and demographic characteristics of the human population and animal reservoir hosts in the transmission of *S. japonicum*. Occurrence of disease in the community should be described in relation to prevalence and intensity of infection.

In the Philippines, schistosomiasis remains endemic in 12 regions covering 28 provinces, 190 municipalities, 15 cities, and 2,222 barangays. Two additional municipalities of Gonzaga, Cagayan (Region 2) and Calatrava, Negros Occidental (Region 6) were recently identified as schistosomiasis endemic areas in 2004 and 2006, respectively, through the identification of indigenous cases, and infected *O. h. quadrasi* snail vector (Figure 5.2). More recent surveys conducted through active surveillance by field schistosomiasis teams revealed a national average prevalence of 2.5% (Table 5.1). The at-risk population is approximately 6.8 million. The highest prevalence of infection is in children 5 to 15 years of age.

A cross-sectional survey done in Western Samar that covered 1,425 households in 50 barangays revealed a schistosomiasis prevalence rate of 18%, with 3.2% having moderate to heavy infection. Epidemiological surveys have demonstrated 10% disease prevalence for Cagayan in 2004 and 69% disease prevalence for Calatrava, Negros Occidental in 2006. A study involving 1,848 school-age children described a resurgence of schistosomiasis in Agusan del Sur with an overall prevalence at 31.8% and proportion of moderate to heavy intensity infections at 19.3%.

Pre-control assessment of the problem of schistosomiasis is essential for evaluation of the effectiveness of control measures. The more useful epidemiologic indices are: (a) prevalence,



Figure 5.2. Map of *Schistosoma japonicum*-endemic provinces in the Philippines

Table 5.1. Prevalence of schistosomiasis stratified by province (2005-2007)

Provinces	Prevalence (%)
Agusan del Sur	3.95
Northern Samar	2.45
Eastern Samar	1.79
Bukidnon	1.66
Surigao del Sur	1.30
Leyte	0.91
Lanao del Norte	0.81
Davao del Norte	0.78
Western Samar	0.77
Compostela Valley	0.68
Mindoro Oriental	0.63
Cotabato – Kidapawan	0.54
Marawi City	0.12
Sorsogon	0.36

Provinces	Prevalence (%)
Surigao del Norte	0.29
South Cotabato	0.28
Sultan Kudarat	0.24
Iloilo City	0.20
Davao del Sur – Digos	0.09
Agusan del Norte	0.08
Cagayan	0.04
Source: Leonardo L, Rivera P, Sanjel O, Villacorte E, Crisostomo B, Hernandez L, et al. Prevalence survey of schistosomiasis in Mindanao and Visayas, The Philippines. Parasitol Int. 2008;57:246-251.	

(b) incidence, and (c) intensity or worm burden estimated according to the number of eggs per unit of weight of feces. It is essential that these indices are determined before the implementation of the control program to have baseline data for evaluation.

The magnitude of the problem is reflected by the prevalence with an expression of the worm burden. Determination of the incidence rate in the younger ages is a more accurate and a more sensitive measure for assessing effects of intervention measures that aim to reduce transmission since schistosomiasis is a chronic infection.

A measurement of worm burden or intensity of infection is done through excretal egg counts. The incidence may not be reduced but the quantum of infective cercariae per exposure may be reduced after therapy so that there is a corresponding decrease in worm burden. In all endemic communities, the distribution of excretal egg count per unit of weight of feces is not normal or random so that a geometric and not an arithmetic mean is a better expression for community egg count. For example, in a study in Irosin, Sorsogon, only a small proportion of the study population (4.1%) excreted 50% of the eggs counted in the study. Excretal egg counts are therefore useful in determining priority of treatment.

Use of mapping of “hot spots” of infection/ transmission by the use of Geographic

Information Systems (GIS) might allow the number of individuals to be monitored for ongoing transmission. New foci of transmission may also be shown reflecting changes in geographical location of transmission foci.

S. japonicum is naturally transmitted between humans and other mammalian hosts, with either humans or animals alone being able to maintain the infection cycle. Prior to application of intervention measures like mass chemotherapy or a program of sanitation, it is important to have a measure of how much of the contamination of the environment with schistosome eggs is attributable to human and animal reservoirs. This will be of value in predicting the success of sanitary disposal of human feces and chemotherapy in reducing transmission and complementary measures of control. The prevalence and egg output should be determined for all possible egg sources.

In the human population, these indices may vary among age groups. Some groups will contribute more than others to contamination. In areas of high prevalence, children aged 5 to 14 years old usually contribute more, whereas in lower prevalence areas, older children and adults are responsible for the bulk of the contamination.

Transmission of infection requires contact between humans and other animal hosts with the breeding sites for snails. As part of pre-control studies, the most common water sites, and the reasons for water contact and their relative importance should be determined and ranked according to relative importance. This should lead to the provision of appropriate alternate facilities (such as protected laundry areas or footbridges) to reduce water contact and determine priorities for snail control.

Prevention and Control

In areas of high prevalence and transmission, mass chemotherapy to reduce morbidity remains the main control strategy. School-age children have been identified as a target group for regular

chemotherapy against schistosomiasis since the WHO Expert Committee on Bilharziasis first met in 1953. Treatment in this age group has been shown to reduce significant morbidity in the short-term and prevent the long-term sequelae in adulthood associated with chronic infection. Continued transmission of schistosomiasis will depend on how rigorously chemotherapy can be applied, as well as on epidemiological factors. In order to achieve a sustainable reduction in transmission, health education, attention to the water supply and sanitation, environmental management, and where appropriate, snail control need to be part of an overall strategy from the very start. The primary objective of chemotherapy using praziquantel is the reduction and prevention of morbidity. Since it is inevitable that prevalence will decrease following treatment, it is important to measure the effect of chemotherapy on incidence, worm burden, and morbidity of new cases. The use of an effective chemotherapeutic agent like praziquantel requires efficient case detection systems and diagnostic tests in order to optimize priorities for treatment where resources will not permit treatment of all infected individuals.

Chemotherapy using praziquantel to reduce morbidity is the principal thrust of the Philippine program for schistosomiasis control. However, it should be stressed that equal emphasis should be placed on control of transmission and eventual elimination of *S. japonicum*, *O. h. quadrasi*, or both, as has been achieved in Japan and in extensive portions of China.

While effective and safe chemotherapy, improved environmental management, and snail control all contribute to the control of schistosomiasis, the long term solution to this problem requires sustained and appropriate health education and strong community participation. Consequently, health education must be recognized as an integral part of the control program. Strong effort should be made

to improve knowledge, attitudes, and perception with respect to transmission, diagnosis, and control of schistosomiasis. Since behavior is influenced by local culture, knowledge, attitudes, and practices (KAP) of the target area should be taken into consideration. This will permit the design of a more applicable and relevant educational program. Health education programs should not only be concerned with modifying KAP but should also encourage and promote community participation in contributing to schistosomiasis control.

O. h. quadrasi is an operculated fresh water amphibious snail (Plate 5.6) with separate male and female sexes. These attain sexual maturity by the time the snails measure 3.5 mm. A single copulation will allow the fertilized female to lay two eggs every 5 days for 1 month. The usual snail habitats are small clear water streams, water-logged rice fields, swamps, and water seepage areas along mountains or foothills. In a stream or small swamp, snails are found both in the water and on the banks. Snails are most numerous in areas where the soil is moist. Those in the water are found in shallower areas, on protruding rocks, or on floating leaves and branches.

Two general strategies for snail control are in use: focal and area-wide. The focal approach

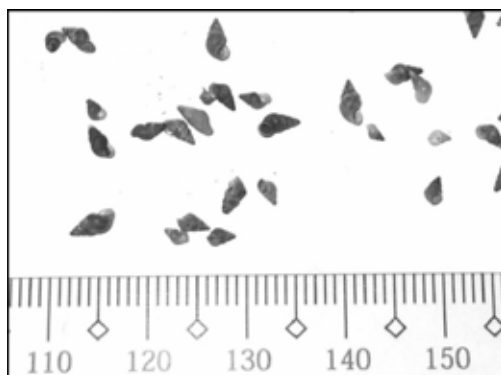


Plate 5.6. *Oncomelania h. quadrasi*, intermediate host of *Schistosoma japonicum* (Courtesy of the Department of Parasitology, UP-CPH)

may be more feasible where transmission sites and resources are limited, but area-wide control is more pragmatic if transmission is spread over a watershed or an irrigation system. Focal control requires water contact studies to identify the most common transmission sites. To control an entire area or watershed unit, all snail habitats should be identified and treated. Area-wide control is more difficult and expensive, but it is also likely to be longer lasting and ultimately more cost-effective than focal measures.

Environmental control methods involve alteration of the snail habitat to reduce survival and prevent or deter snail reproduction. Control of breeding has a more lasting effect than killing snails. The more radical the intervention, the more profound the effect of the control measure on the snail population. Methods of control are based on removal of the environmental requirements of *Oncomelania*. These include: (a) drainage of breeding sites and proper management of irrigation systems; (b) removal of shade or shelter from the sun by clearing vegetation around bodies of water; (c) prevention of breeding on the banks of streams or irrigation canals by lining these with concrete or making them more perpendicular; (d) acceleration of flow of water by proper grading and cleaning of the stream bed and removal of debris; (e) construction of ponds if the area cannot be drained; and (f) covering snail habitats with landfills.

The effectiveness of these alterations is lasting if there is proper maintenance. Although snail control is usually done on a focal basis, when possible, it should include entire watershed. All of these methods have been found to be effective experimentally as early as 1958 in the Philippines. One of the rate limiting factors of environmental modification of habitat is the cost involved. Japan was able to afford the large capital expenditure needed for cementing canals, reclaiming swampy areas, and sustaining the control program. In China, the socio-political structure made it possible to

implement the necessary environmental changes without resorting to large capital expenditure. In the Philippines where there is a perennial shortage of funds, increased community participation is needed to ensure the success of snail control programs. The advantages of snail control by environmental methods include the following: (a) it can be incorporated or integrated into regional agricultural and other rural development projects; (b) the results can be made permanent or persistent provided adequate maintenance is done regularly; (c) it results in increased agricultural productivity; (d) in the absence of adequate funding, the control measures can be done on a focal basis by the people themselves; (e) it results in increased land value; and (f) it does not require foreign aid and technology, unlike chemical control.

No outstanding novel molluscicide or chemical for killing snails has been developed in the past decade. Interest in such research by industry has diminished because of high research and development cost and the lack of an assured market. Most countries that have schistosomiasis cannot afford the cost of deploying molluscicides, and there is increasing concern about the consequent environmental pollution with pesticides that are not biodegradable or have long half-lives. The future role of molluscicides may be determined by economic considerations and the priority afforded schistosomiasis in relation to other public health problems.

The objective of sanitary disposal of human feces is to prevent contamination of watercourses inhabited by snails. However, this has limited value in *S. japonicum* transmission if animal reservoir hosts represent a significant source of miracidia for infecting snails.

The use of properly constructed and hygienic latrines should be encouraged as this contributes to the control of water and fecal-borne viral, bacterial, and parasitic infections. Latrines for use in rural areas have been regarded as unsatisfactory because of flies, mosquitoes,

and maintenance problems. These issues should be resolved to increase toilet utilization.

The simplicity of diagnostic techniques, the safety of praziquantel, the relative facility of focal control of snails, and the availability of epidemiologic information for some endemic areas permit adoption and integration of schistosomiasis control into primary health care. This stimulates active involvement of the community and facilitates the entry into endemic communities of support services and schistosomiasis teams of the Department of Health.

Primary health care workers in endemic areas should have some basic knowledge of schistosomiasis, including major clinical manifestations, method of diagnosis, treatment, transmission, and control. They should be involved in stool collection, surveys, and treatment of patients. They should also be utilized as health educators, and asked to encourage community participation, particularly in sanitation and snail control.

The effectiveness of intervention measures must be monitored and evaluated to ensure that the approaches are effective and properly implemented to detect any resurgence, and to justify costs. The thrust and components of the control program determine the indices that will be measured as basis for evaluation. It is essential to have adequate baseline data, especially for pilot areas, prior to the implementation of control operations to allow for adequate assessment.

Control programs should have operational targets and corresponding timetables for each endemic area. For example, there should be targets for social preparation of the population for awareness building and participation. The coverage and timetable for parasitologic examination and chemotherapy should be ascertained. Snail colonies or areas to be treated should be identified. Equally important is the standardization of techniques to be used. Monitoring and supervision should ensure

operational efficiency so that evaluation of the effects of control operations will be valid and will truly reflect the epidemiologic profile of the disease.

A transmission blocking vaccine has been developed for water buffaloes in China and represents a major breakthrough in controlling animal reservoirs. However, domesticated animals seem to be the minority reservoir in the Philippines in comparison to sylvan reservoirs and human sources of infection. Development of a human vaccine has proven difficult since *Schistosoma* is well-adapted to evading the immune system in its niche as an intravascular parasite. Several parasite antigens are promising vaccine candidates, including paramyosin, which has generated immunity to repeated infection in pilot studies. The mapping of the schistosome genome will enable the identification of more vaccine candidate molecules and other possible novel mechanisms for the treatment and control of this parasite.

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Lung Flukes

Vicente Y. Belizario, Jr., Alexander H. Tuliao

Paragonimus westermani

Paragonimiasis is an infection of humans and other mammals by trematodes of the genus *Paragonimus*. There are 40 known species of *Paragonimus*, and six are reported to cause infections in humans. *Paragonimus westermani* or the Oriental lung fluke causes 90% of paragonimiasis in humans. In the Philippines, *P. westermani* is also the major species that causes paragonimiasis in humans. The only other species in the Philippines is *P. siamensis*, which has only been identified in cats.

In 1879, Ringer observed the first case of pulmonary paragonimiasis in humans during an autopsy in Formosa. A year later, Baelz (1880) in Japan and Manson (1880) in Formosa identified *Paragonimus* ova in human sputum. Musgrave (1907) described the first case of human paragonimiasis in the Philippines.

In 1915, Nakagawa discovered that crabs act as a second intermediate host. Two years later, Nakagawa succeeded in infecting the snail *Melania libertine* with *Paragonimus* miracidia.

Parasite Biology

The adult lung fluke (Plate 5.7) is reddish-brown and measures 7 to 12 mm in length, 4 to 6 mm in width, 3.5 to 5 mm in thickness, and resembles a coffee bean. It is rounded anteriorly and slightly tapered posteriorly. The tegument is covered with single-spaced spines. The two testes are deeply lobed and are situated opposite each other, almost midway between the ventral sucker and the posterior border of the body. The ovary is located anterior to the testes and posterior to the ventral sucker, and has six long unbranched lobes. The vitellaria are branched extensively.

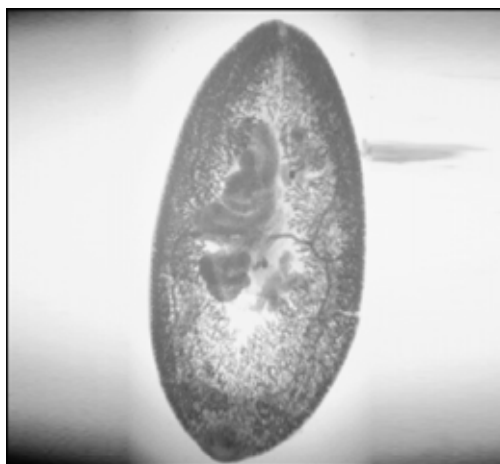


Plate 5.7. *Paragonimus westermani* adult
(Courtesy of the Department of Parasitology,
UP-CPH)

The cercaria is covered with spines, has an ellipsoidal body, and a small tail. A stylet is present at the dorsal side of the oral sucker. The metacercaria is round and measures from 381 to 457 μm . The oval, yellowish-brown, thick-shelled egg measures 80 to 118 μm by 48 to 60 μm , and has a flattened but prominent operculum. Opposite the operculum is a thickened abopercular portion (Plate 5.8). It is unembryonated at oviposition.

The immature egg embryonates in water, moist soil, or leached feces (Figure 5.3). A miracidium develops within 2 to 7 weeks. It subsequently pushes open the operculum and swims freely in search of its appropriate snail host. In the Philippines, the 1st intermediate hosts are *Antemelania asperata* and *Antemelania dactylus*, the former previously known as *Brotia asperata* (Plate 5.9). Inside the snail, the miracidium passes through one sporocyst and two redial stages of development. Cercariae



Plate 5.8. *Paragonimus westermani* egg; note the flattened operculum and the abopercular portion (Courtesy of the Department of Parasitology, UP-CPH)

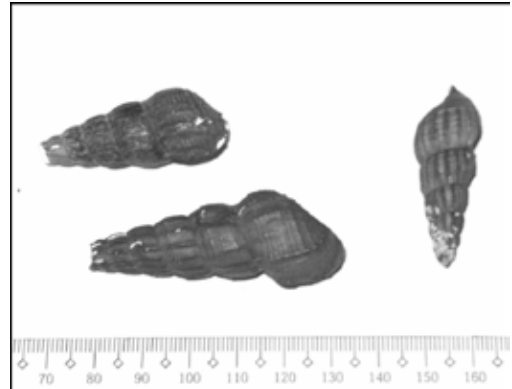


Plate 5.9. *Antemelania asperata*, first intermediate host of *Paragonimus westermani* (Courtesy of the Department of Parasitology, UP-CPH)

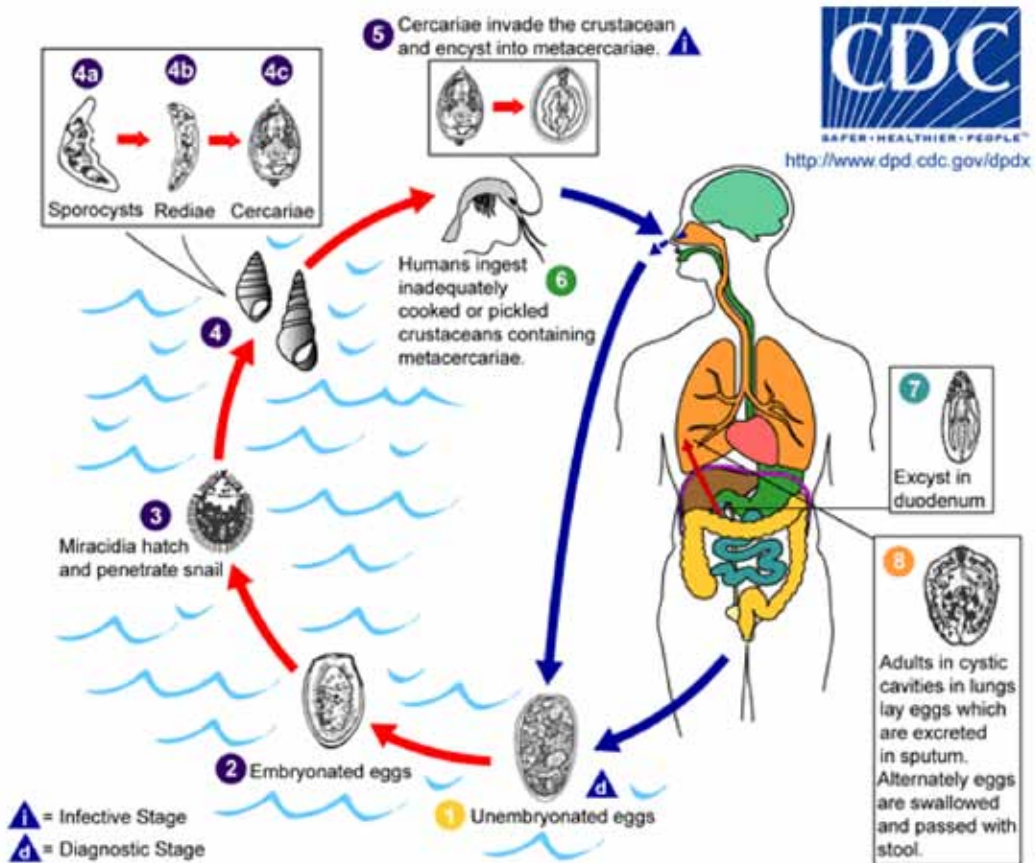


Figure 5.3. Life cycle of *Paragonimus westermani* (Accessed from www.dpd.cdc.gov/dpdx)

subsequently emerge from the snail to seek and infect the second intermediate host, the mountain crab *Sundathelphusa philippina* (Plate 5.10), formerly known as *Parathelphusa grapsoides*. The cercaria penetrates the soft parts of the crustacean and encysts as a metacercaria in the gills, body muscles, viscera or legs (Plate 5.11). The crab may also be infected by eating infected snails. The definitive host acquires the infection by ingesting raw or insufficiently cooked crabs harboring metacercariae.

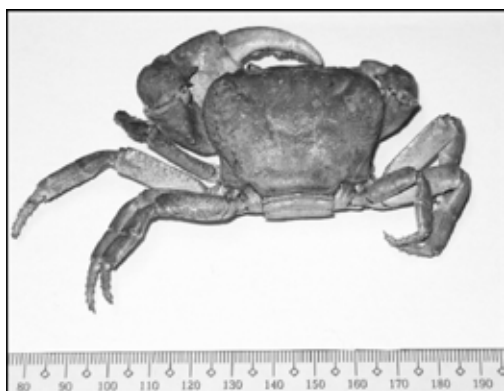


Plate 5.10. *Sundathelphusa philippina*, the second intermediate host of *Paragonimus westermani* (Courtesy of the Department of Parasitology, UP-CPH)

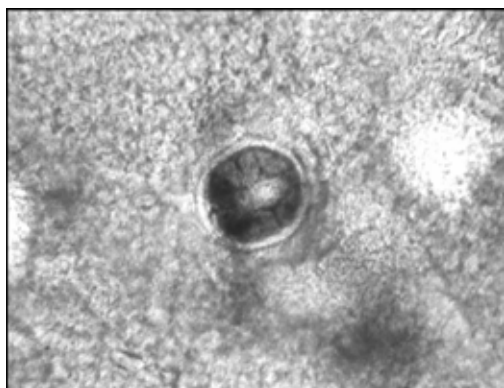


Plate 5.11. *Paragonimus westermani* metacercaria in crab heart muscle (Courtesy of the Department of Parasitology, UP-CPH)

Following the ingestion of infected crustacean tissue by the host, the metacercariae of *Paragonimus* excyst in the duodenum of the host. The immature worm then traverses through the intestinal wall into the peritoneal cavity, where it wanders about for several days and embeds itself in the abdominal wall. The parasite then returns to the coelom and migrates through the diaphragm into the pleural cavity. A juvenile diploid worm wanders in the pleural spaces until it finds one or several diploid worms. The pair or group then migrates into the lung parenchyma to develop into adults in about 6 weeks, where they mate and lay eggs. Juvenile triploid worms in Japan, Korea, and Taiwan can establish themselves in the lung parenchyma without a mate. Groups of diploid and triploid parasites have also been observed.

In the lung parenchyma, a fibrotic capsule forms around the parasite or their group. The fibrotic capsule has openings that allow the eggs to escape into the respiratory tract where they are moved up and out by the ciliary epithelium along with lung exudates. In the pharynx, they are either coughed out or swallowed into the alimentary canal to be passed out with the feces. The completion of development in the definitive host takes 65 to 90 days. Adult worms are known to persist in humans for 20 years or longer.

Cysteine proteases have been found to play an important role in the development of young parasites because of their involvement in the metacercarial excystment, tissue invasion, and immune modulation of the host. Cysteine proteases with masses of 27 and 28 kD are released from the excretory bladder of the metacercariae during excystment. The proteases are most abundant in the tegmentum of newly excysted worms, facilitating migration through the tissues of the host. The 27 and 28 kD cysteine proteases are also found to cleave human immunoglobulin G, thereby creating a zone of immune privilege around the worm. As the juvenile parasite moves actively towards

the lungs, additional proteases of 15, 17, and 53 kD are expressed. Protease activity decreases as worm matures.

Pathogenesis and Clinical Manifestations

In the lungs, *Paragonimus* worms provoke a granulomatous reaction that gradually gives rise to the development of a fibrotic cyst containing blood-tinged purulent material, adult worms, and eggs. The most common symptoms of paragonimiasis are chronic cough and hemoptysis. Chest pain, dyspnea, low-grade fever, fatigue, and generalized myalgia may also occur.

Since it takes several weeks for the parasite to migrate and mature, the early stages of the infection are usually asymptomatic. Clinical symptoms are less severe after 5 to 6 years. Occasionally, the disease can have serious sequelae, such as chronic bronchiectasis and pleural fibrosis, secondary to severe parenchymal and pleural damage.

The circuitous route of migration allows the worms to lodge and mature in different ectopic locations. These aberrant worms may localize in the lung pleura, pericardium, myocardium, abdominal wall, omentum, liver, mesenteric lymph nodes, adrenals, urogenital organs, and eyes. Heavy intensity infections can cause both pulmonary and ectopic paragonimiasis. Worms that fail to find a mate in low intensity infections may end up in ectopic locations as well.

Cutaneous and cerebral paragonimiasis are the classic known forms of ectopic infection. In cases of cutaneous paragonimiasis, a slow-moving, nodular lesion in the subcutaneous tissue on the abdomen or chest is the characteristic symptom.

Cerebral involvement is the most serious complication of human paragonimiasis. A juvenile *P. westermani* may migrate from the pleural cavity into the cranial cavity through the soft tissues along the internal jugular vein. The migration worm can cause congestion, vasculitis, and capillary rupture, which may

result in exudative aseptic inflammation, infarction, hemorrhage, and necrosis in the subcortical areas. After invasion, multiple, conglomerated, and interconnected granulomas form around the parasite, containing abscess material and eggs. In the chronic stage, liquefaction necrosis and fibrinous gliosis occur, and these may lead to cortical or subcortical atrophy, and secondary ventricular dilatation. Cerebral paragonimiasis may present with headache, meningismus, seizures, hemiparesis, blurring of vision, diplopia, homonymous hemianopsia, and aphasia.

Diagnosis

Microscopy is the most basic and most readily available diagnostic tool for paragonimiasis. Definitive diagnosis is based on the detection of the characteristic eggs in sputum, stool, or, less frequently, in aspirated material from abscesses or pleural effusions. However, the sensitivity of microscopy is suboptimal, with ova detection in sputum ranging from 37 to 88%. If initial findings are negative, repeat examinations may be helpful. Sputum concentration with 3% sodium hydroxide, with repeated sputum examinations up to three times on different days, provides the best sensitivity for microscopic diagnosis.

Chest radiographs may aid in the diagnosis of pulmonary paragonimiasis when combined with a high index of suspicion. Pulmonary paragonimiasis usually presents as lung parenchyma lesions which may be infiltrative, nodular, cavitating, or a combination of these. Pleural effusions occur in almost half of patients. These radiographic findings are not specific, and may also be seen in other diseases, particularly pulmonary tuberculosis (PTB), lung cancer, and fungal infections. Since PTB and paragonimiasis are usually co-endemic, PTB should always be ruled out.

The peripheral blood count for paragonimiasis frequently reveals eosinophilia and elevated levels of IgE, which is typical for

parasitic infections. The total white blood cell count may be in the normal to elevated range. Eosinophilia is more common in the acute stage of paragonimiasis, whereas IgE levels have no correlation with the stage of the disease.

Various immunological methods have been developed for the diagnosis of paragonimiasis. Classic methods include the complement fixation (CF) test, intradermal test, double diffusion in agarose gel, and immunoelectrophoresis. CF has high sensitivity for both diagnosis and assessment of cure after therapy. The intradermal test is simple, rapid, cheap and highly sensitive, although it may still yield positive results several years after successful treatment.

The classic methods for serodiagnosis of paragonimiasis have been gradually replaced by more sensitive and specific tests, like immunoblotting (IB) and enzyme-linked immunosorbent assay (ELISA). IB has a sensitivity of up to 99%, and has been used since 1988. ELISA has a sensitivity ranging from 96% to 99%, and has been employed widely in various parasitic and non-parasitic infections. For paragonimiasis, most ELISA systems were developed to detect *Paragonimus*-specific IgG antibody. Attempts have also been made to detect specific IgE, IgM, and circulating antigens. The multiple-dot ELISA was developed for field use in developing countries.

The loop-mediated isothermal amplification (LAMP) test is a simple, rapid, and cost-effective method currently being developed for field use in epidemiologic surveys in developing countries. LAMP allows the rapid amplification of deoxyribonucleic acid (DNA) with high specificity under isothermal conditions, using DNA polymerase with strand-displacement activity. Magnesium pyrophosphate, the reaction by-product, is visible to the naked eye. Only warm water is required to perform the assay.

In cerebral paragonimiasis, the most characteristic finding in either cranial Computer Tomography (CT) scan or Magnetic Resonance Imaging (MRI) are conglomerated, multiple, ring-enhancing lesions (“grape-cluster” appearance) with surrounding edema, typically in one cerebral hemisphere, most commonly in the posterior part of the brain. On skull radiographs, patients with chronic disease may present with specific soap-bubble calcifications.

Treatment

Praziquantel is the drug of choice. It is highly effective in the treatment of trematode infections, particularly lung fluke infection. It induces rapid contraction of trematodes and alters the tegmental surface (e.g., vacuolization). These changes are thought to be linked to the drug-dependent disruption of calcium homeostasis. Praziquantel is suitable for treatment of adults and children over 4 years of age. Usual dose for treatment is 25 mg/kg, three times a day, for 2 to 3 days. A higher dose may be required in cases of ectopic paragonimiasis.

Praziquantel is currently not recommended for the treatment of paragonimiasis during pregnancy and lactation, although current literature has not proven the drug to have mutagenic, teratogenic, or embryotoxic effects. Treatment should preferably be given after delivery unless immediate intervention is deemed essential. Breastfeeding should be avoided during and 72 hours after treatment. Adverse effects of praziquantel are generally mild, and these include abdominal discomfort, nausea, headache, dizziness, and rarely, fever, urticaria, drowsiness, and tachycardia.

Triclabendazole is a benzimidazole that was originally used for the treatment for *Fasciola hepatica* infections. Recently, triclabendazole has been demonstrated to be an effective drug against human paragonimiasis. Triclabendazole probably binds to B-tubulins of trematodes, leading to depolymerization and disruption of

microtubule-based processes. These result in damage to the external plasma membrane and nuclear membrane, with dissolution of some heterochromatin, mitochondria, and Golgi complex. The cure rate with triclabendazole is comparable with that of praziquantel, and may result in better patient compliance since the treatment regimen consists only of a single dose.

Bithionol can be used as an alternative drug. It is given orally at a dose of 15 to 25 mg/kg, twice daily on alternate days, for 10 to 15 days.

Epidemiology

Paragonimiasis has a focal distribution in limited parts of Asia, Latin America (Peru and Ecuador), and Africa (Nigeria and Cameroon). According to recent estimates, 20.7 million people worldwide are infected, and 292.8 million are at risk.

In the Philippines, paragonimiasis is endemic in Mindoro, Camarines, Sorsogon, Leyte, Samar, Zamboanga del Norte, Davao Oriental, Basilan, and Cotabato. Prevalence rates vary among the endemic provinces. Infection rates in Sorsogon ranged from 16 to 25% in 1997. In more recent epidemiologic studies done in the municipality of Pres. Manuel Roxas in Zamboanga del Norte, the prevalence was 14.8% in 2005.

Paragonimiasis has a focal distribution, largely determined by local patterns of consumption of inadequately cooked crustaceans and paratenic hosts. Examples of dishes that can transmit disease include *kinagang* (crab in coconut milk), *sinugba* (grilled crab), and *kinilaw* (raw crabs in vinegar) in the Philippines, *nam prik poo* (crab and chilli paste) in Thailand, crabs in brine, soy sauce or alcohol (drunken crabs) in China, *kejang* (raw crabs in soy sauce) in Korea, *ceviche* (raw crabs in lemon sauce) in Peru, and *sashimi* of wild boar and bear

meat in Japan. Unhygienic food preparation also contributes to the transmission of the disease.

Cultural beliefs and traditions influence the age and sex distribution of paragonimiasis. In Japan, during the 1950s and 1960s, the majority of those infected were children because of the practice of using raw crayfish juice as a treatment for various cutaneous ailments. Similar practices also existed in Korea during the same period. Currently, middle-age Japanese men have the highest prevalence due to their conservative affinity for traditional dishes. In adolescent girls in Cameroon, a popular belief existed once among the Bakossi people that crabs aid in fertility, leading to disproportionately high infection rates in this group.

PTB overlaps with paragonimiasis in paragonimiasis endemic areas in the Philippines and other developing countries. Since PTB and pulmonary paragonimiasis share the same symptoms, misdiagnosis and mismanagement are not uncommon. Further studies are needed to elucidate the impact of misdiagnosis of pulmonary paragonimiasis and PTB.

Prevention and Control

The most practical way to prevent acquisition of human paragonimiasis is to avoid ingestion of raw or insufficiently cooked crabs and other crustaceans, as well as meat from paratenic hosts like wild pigs. Safe food preparation helps reduce the infectivity of food. Furthermore, it is believed that changing the risky dietary habits of the population, through health education and promotion, can control this parasitic infection. Elimination of reservoir and intermediate hosts of *Paragonimus* may not be feasible. Capacity building of local health staff on the diagnosis and treatment of this disease is important for early case detection and treatment.

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Intestinal Flukes

Vicente Y. Belizario, Jr., Percy G. Balderia

Fasciolopsis buski

This fasciolid digenetic trematode is the largest intestinal fluke of humans and pigs.

Parasite Biology

The mode of transmission of *Fasciolopsis buski* is by ingestion of encysted metacercariae from aquatic plants. This can occur when the plant itself is eaten, or when the hull or skin of the fruits of these plants is peeled off between the teeth. The viable metacercaria excysts in the

duodenum and attaches to the intestinal wall, where it becomes sexually mature in about 3 months. The adult worm lives in the duodenum, attached to the intestinal mucosa by its suckers (Figure 5.4). In heavy infections, the worms may be found throughout the intestinal tract. Immature eggs are released together with feces into the water. The egg, which embryonates in water, gives rise to a miracidium in 3 to 7 weeks. The miracidium then seeks out and infects its first intermediate host, a snail belonging to either the genus *Segmentina* or *Hippeutis*.

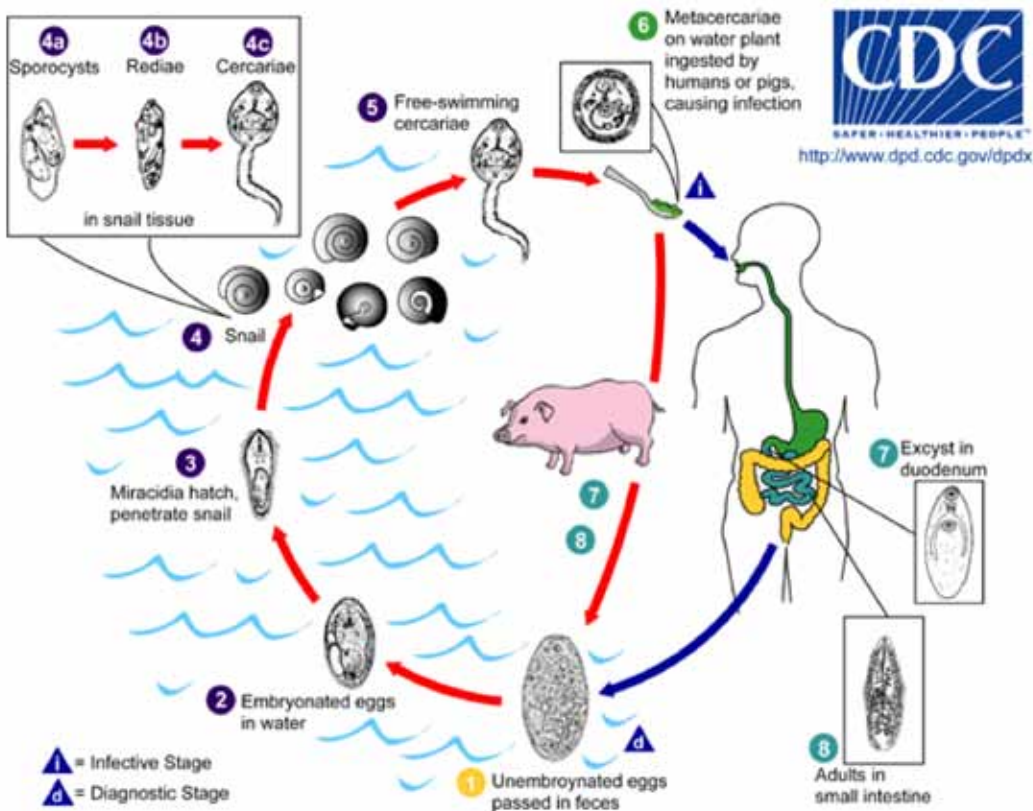


Figure 5.4. Life cycle of *Fasciolopsis buski*
(Accessed from www.dpd.cdc.gov/dpdx)

Inside the snail, the miracidium transforms into a sporocyst, which subsequently produces mother rediae, daughter rediae, and finally, cercariae. Cercariae leave the daughter rediae and undergo further development in the snail tissues. Seven weeks after infection, cercariae emerge from the snails into water. Cercariae attach themselves and encyst as metacercariae on the surfaces of seed pods, bulbs, stems, or roots of various aquatic plants such as *Trapa bicornis* (water caltrop) (Plate 5.12), *Eliocharis tuberosa* (water chestnut), *Ipomea obscura* (morning glory or kangkong), and *Nymphaea lotus* (lotus). These plants serve as the second intermediate hosts of the parasite. Pigs and humans are the important definitive hosts.

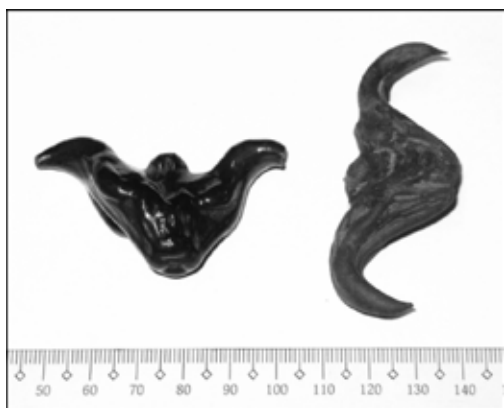


Plate 5.12. *Trapa bicornis*, second intermediate host of *Fasciolopsis buski* (Courtesy of the Department of Parasitology, UP-CPH)

F. buski is elongated, oval in shape, and measures 20 to 75 mm in length, and 8 to 20 mm in width. Compared to *Fasciola hepatica* and *F. gigantica*, it does not have a cephalic cone, and its intestinal ceca are unbranched and reach up to the posterior end. The two testes are dendritic, and are arranged in tandem in the posterior half of the body. The branched ovary lies to the right of the midline. Fine vitelline follicles are situated throughout the lateral margin of the body.

The egg is large, operculated, unembryonated when first passed, and indistinguishable from eggs of *F. hepatica* and *F. gigantica*. It measures 130 to 140 μm by 80 to 85 μm .

Pathogenesis and Clinical Manifestations

Pathological changes caused by the worms are traumatic, obstructive, and toxic. Inflammation and ulceration occur at the site of worm attachment, producing an increase in mucus secretion, and minimal bleeding. Gland abscesses are occasionally formed in the mucosa. In heavy infections, the worms may cause intestinal obstruction. Intoxication results from the absorption of worm metabolites by the host. The patient experiences generalized toxic and allergic symptoms, such as edema of the face, abdominal wall, and lower limbs. Profound intoxication can result in death of the host.

Diagnosis

Diagnosis is by detection of parasite eggs in the stool. *Fasciolopsis buski* eggs resemble *Fasciola* eggs under the microscope.

Treatment

Praziquantel is given in three doses of 25 mg/kg over 1 day. Minimal side effects are associated with the drug. There may be episodes of epigastric pain, dizziness, and drowsiness, which typically disappear within 48 hours. In a study carried out in Central Thailand, 100% cure rates were demonstrated for regimens of 15, 25, and 40 mg praziquantel per kg body weight. Until further studies show the efficacy of the 15 mg/kg regimen, the authors still recommend a dose of 25 mg/kg for the treatment of fasciolopsiasis.

Epidemiology

Fasciolopsiasis is endemic in the countries of Southeast Asia, China, Korea, and India. Its endemicity in the Philippines has not yet been demonstrated. No locally acquired fasciolopsiasis in humans or pigs has been

reported. Fasciolopsiasis in Filipinos were probably acquired abroad.

Prevention and Control

Since metacercariae are very sensitive to dryness, soaking of aquatic plants in water should be avoided. The time between harvest

and consumption could also be prolonged to prevent infection. Washing of the plants to remove metacercariae, or boiling them to kill the parasites can also prevent infection. Swamps or ponds where aquatic plants are cultivated should be protected from pollution by untreated human or pig excreta.

Echinostoma ilocanum *Artyfechinostomum malayanum*

The echinostomids are digenetic trematodes characterized by a collar of spines around their oral suckers. There are several species which infect humans. Two species have been documented in the Philippines.

Parasite Biology

The mode of transmission of *Echinostoma ilocanum* and *Artyfechinostomum malayanum* is by ingestion of metacercariae encysted in snails, the second intermediate hosts of the parasites. When the metacercariae reach the duodenum, they excyst and the juvenile fluke attaches to the

wall of the small intestine, where they develop into sexually mature adult worms.

The adult worms live in the small intestine of the definitive host (e.g., humans, dogs, cats, rats, and pigs). Immature eggs released by the parasite are transported to the environment with the feces. The egg matures in water, and after 6 to 15 days, a miracidium hatches from the egg to infect the first snail intermediate host. Inside the snail, the *E. ilocanum* miracidium develops into mother rediae, which subsequently produce daughter rediae and cercariae after 42 to 50 days. The *A. malayanum* miracidium first

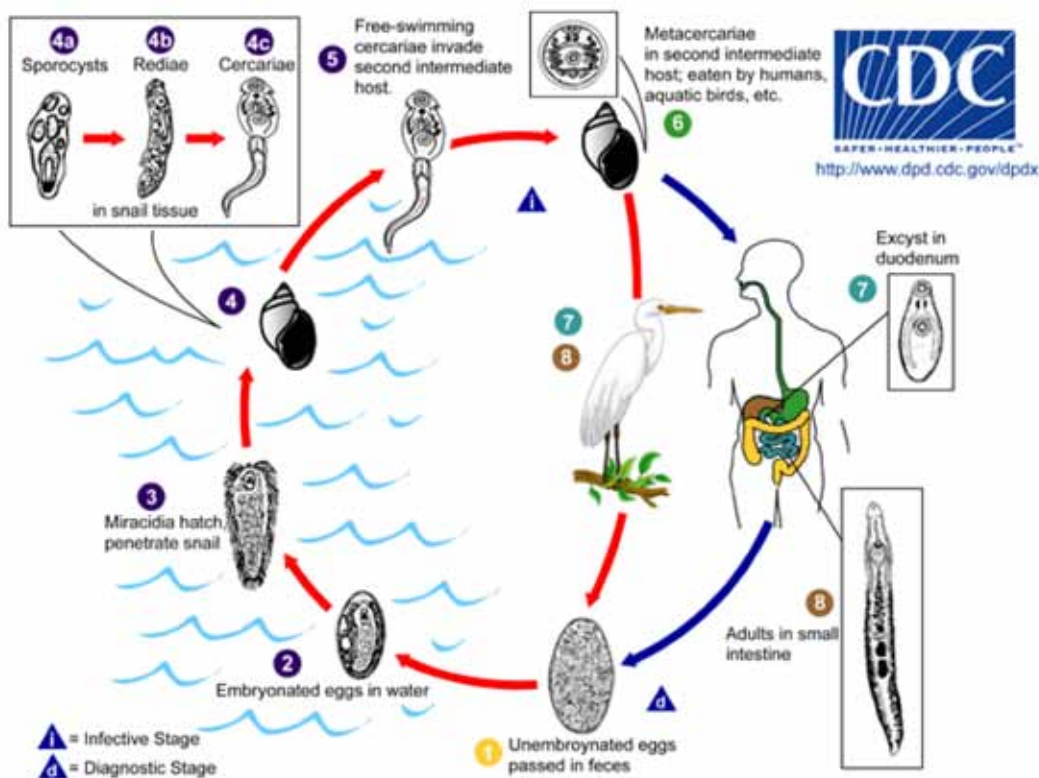


Figure 5.5. Life cycle of *Echinostoma* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

develops into a sporocyst, which subsequently produces mother rediae, daughter rediae, and cercariae. After escaping from the snail, cercariae swim in water to seek out and infect the second snail intermediate host, in which they transform into metacercariae. The metacercaria is the infective stage to the definitive host (Figure 5.5).

In the Philippines, the first snail intermediate host species of *E. ilocanum* are *Gyraulus convexiusculus* and *Hippeutis umbilicalis*. The second snail intermediate hosts are *Pila luzonica* (*kuhol*) (Plate 5.13) and *Vivipara angularis* (*susong pampang*). The first snail intermediate host species of *A. malayanum* in the Philippines has not yet been identified, but is suspected to be the same as that of *E. ilocanum*. However, it has been confirmed that the second snail intermediate hosts are either *Lymnaea* (syn. *Bullastra*) *cumingiana* (*birabid*) or *Ampullarius canaliculatus* (golden apple snail).



Plate 5.13. *Pila luzonica*, second intermediate host of *Echinostoma ilocanum*
(Courtesy of the Department of Parasitology, UP-CPH)

E. ilocanum (Plate 5.14) is reddish-gray and measures 2.5 to 6.6 mm in length and 1 to 1.35 mm in width. The worm is tapered at the posterior end and has 49 to 51 collar spines. The oral sucker lies in the center of the circumoral disk, and the ventral sucker is situated at the anterior fifth of the body. The two testes are deeply bilobed, arranged in tandem in the third

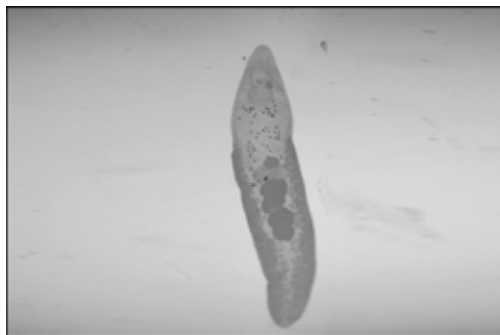


Plate 5.14. *Echinostoma ilocanum* adult
(Courtesy of the Department of Parasitology, UP-CPH)

quarter of the body. The ovary is located just in front of the anterior testis. Follicular vitellaria are located in the posterior half of the body, and uterine coils are found between the ovary and the ventral sucker. The intestinal ceca are simple.

A. malayanum measures 5 to 12 mm in length and 2 to 3 mm in width. It has a rounded posterior end and has 43 to 45 collar spines. The two testes are large, each with six to nine lobes arranged in tandem. The ovary is small, rounded or oval, located anterior to the testes, and pre-equatorial (Plate 5.15).

The *E. ilocanum* egg is straw-colored, operculated, and ovoid, measuring 83 to 116 μm by 58 to 69 μm , whereas the *A. malayanum* egg is larger, golden brown in color, operculated, and measures 120 to 130 μm by 80 to 90 μm .

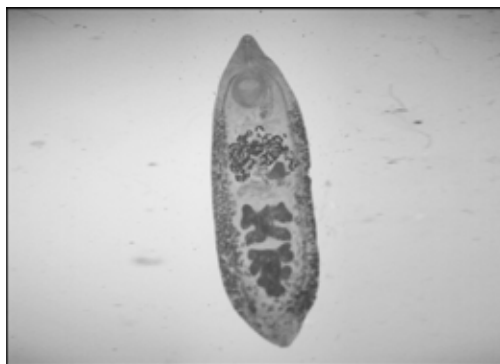


Plate 5.15. *Artyechinostomum malayanum* adult
(Courtesy of the Department of Parasitology, UP-CPH)

Pathogenesis and Clinical Manifestations

In heavy infections, inflammation develops at the site of attachment of the adult worm to the intestinal wall. Ulceration, and consequently, diarrhea, which is sometimes bloody, as well as abdominal pain may also develop. The absorption of metabolites from the worms may result in general intoxication.

Diagnosis

Diagnosis is by detection of eggs in the stool. Notably, the eggs of echinostomes, *Fasciola*, and *Fasciolopsis buski* look very much alike, although the latter two are bigger in size.

Treatment

Three doses of praziquantel may be given at 25 mg/kg per dose over 1 day.

Epidemiology

These two parasites have been reported in other Southeast Asian and East Asian countries.

The endemicity of both parasites is related to the eating habits of the population. *E. ilocanum* infection is endemic in Northern Luzon, Leyte, Samar, and the provinces of Mindanao. *A. malayanum* infection in the Philippines was first documented in humans in 1987, and has since been reported in Northern and Central Luzon. In 2005, a study in Siargao Island, Surigao del Norte, showed *A. malayanum* in 11.4% of individuals suffering from gastrointestinal disturbance. All infected patients had a history of having eaten snails (*kuhol* and *kiambuay*) prepared raw with coconut milk and lime juice.

The second snail intermediate hosts are abundant in rice fields especially during the wet months. The rat is probably an important reservoir host of both echinostomes.

Prevention and Control

Preventive measures involve mainly avoiding ingestion of raw or improperly cooked second intermediate snail hosts of these parasites.

Heterophyid Flukes

There are many species of heterophyids that live in the intestines of fish-eating hosts. The major species are *Heterophyes heterophyes*, *Metagonimus yokogawai*, *Haplorchis taichui*, and *Haplorchis yokogawai*.

Parasite Biology

The mode of transmission of heterophyids is by ingestion of metacercariae encysted in fish (Figure 5.6). When the metacercariae reach the duodenum, they excyst, liberating young larvae that attach to the intestinal wall. The larvae subsequently develop into sexually mature adult worms that have a typically short life span of less than 1 year.

The adult worm inhabits the small intestine of the definitive host. Large numbers of eggs are produced and passed out into the environment together with feces. The eggs hatch into miracidia after ingestion by the first snail intermediate host. Inside the snail, the miracidia develop further into sporocysts, which eventually develop into one or two generations of rediae that give rise to cercariae.

Cercariae that are liberated from the snail encyst as metacercariae on or under the scales, in the muscles, fins, tails, or gills of fish species that serve as second intermediate hosts. Metacercariae are frequently found in the muscles at the base of the fin.

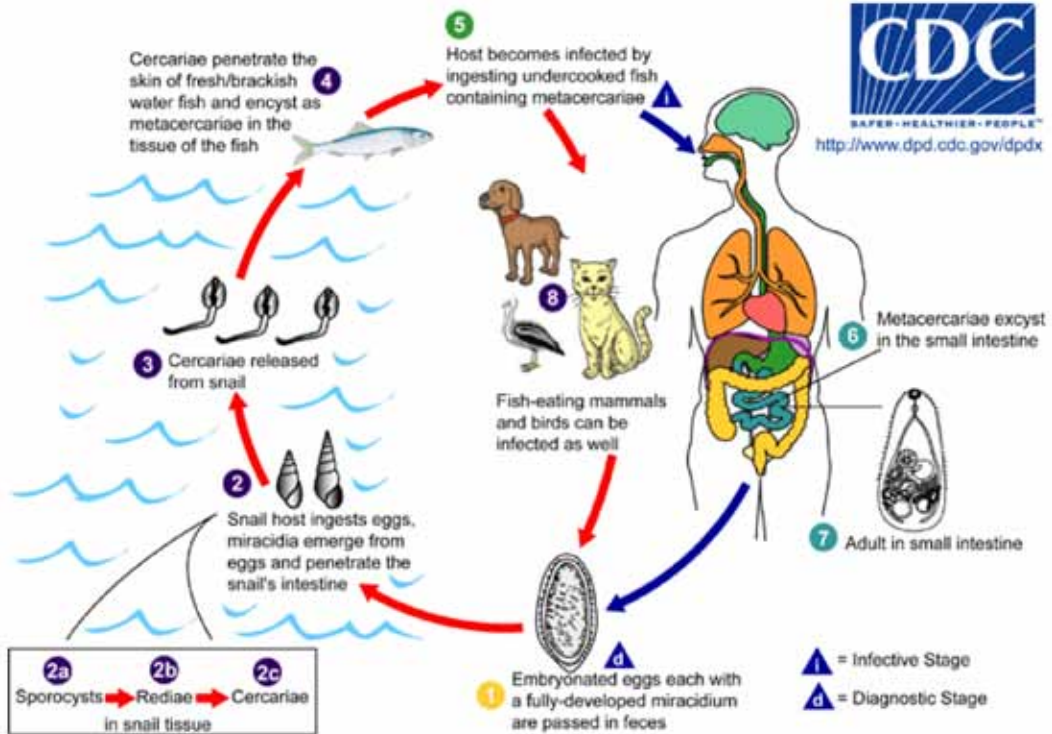


Figure 5.6. Life cycle of heterophyids
(Accessed from www.dpd.cdc.gov/dpdx)

The snail hosts can be freshwater, brackish water, or marine species. In the Philippines, the snail hosts of *H. taichui* and *Procerovum calderoni* are the brackish water snails, *Melania juncea*, and *Thiara riquetti*, respectively. The local snail intermediate host species of other heterophyid parasites have not yet been identified.

In the Philippines, there are at least 30 known species of fish harboring metacercariae of 21 heterophyid species (Table 8.2). The adult fluke is elongated, oval or pyriform, and it measures less than 2 mm in length. The tegument has fine scale-like spines. Some species have a gonotyl or a genital sucker that is located near the left posterior border of the ventral sucker. Testes, variously arranged, are in the posterior end of the body; and the ovary, globular or slightly lobed, is located in the submedian, pre- or post-testicular area (Plate 5.16).

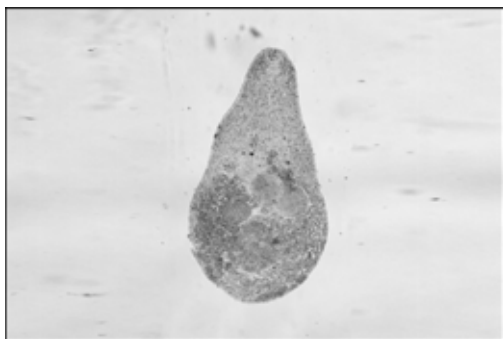


Plate 5.16. Heterophyid fluke adult
(Courtesy of the Department of Parasitology,
UP-CPH)

The egg is light brown in color, ovoid in shape, operculated, and measures 20 to 30 μm by 15 to 17 μm (Plate 5.17). A fully developed, symmetrical miracidium is already present within the egg when it is deposited by the adult worm. The operculum fits into the eggshell smoothly, and it does not have an abopercular protruberance, in contrast to *Clonorchis* and *Opisthorchis* eggs.

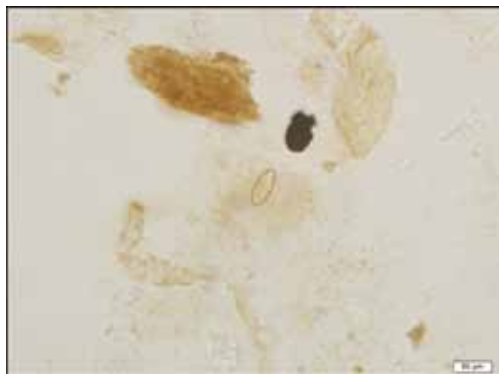


Plate 5.17. Heterophyid egg
(Courtesy of Prof. Winifreda U. de Leon)

Pathogenesis and Clinical Manifestations

There is usually inflammation at the sites where the worm is attached to or burrowed in the mucosa. Excessive mucus production and sloughing off of the superficial layers may occur. In a study done in Compostela Valley in Southern Mindanao, the most common clinical manifestations observed were consistent with peptic ulcer disease (PUD) or acid peptic disease (APD). These included upper abdominal discomfort/pain, reported by 42.2% of patients, and gurgling abdomen, which was found in 24.1% of patients. Colicky abdominal pain and mucoid diarrhea may also be present.

A report by Africa in 1931 showed that worms tend to burrow deep into the intestinal wall, where they become trapped and eventually die. Eggs of degenerating worms may be filtered through the intestinal lymphatics and blood vessels and may be deposited in various tissues. Eggs and adults of heterophyids have been observed in the heart and brain of Filipino patients who died of heart failure and intracerebral hemorrhage. Eggs lodged in the spinal cord may result in sensory and motor losses at the level of the lesion.

Diagnosis

Considering the similarity in presentation of heterophyidiasis with APD, it is important

to consider intestinal fluke infection when dealing with bowel disturbance and a history of consumption of raw fish. Definitive diagnosis is by detection of eggs in the stool using the modified Kato thick method, which has a higher sensitivity compared to formalin-ether/ethyl acetate concentration technique (31.0% vs. 13.6%). The eggs of the different heterophyid species are difficult to distinguish. Care must be taken to distinguish them from *Clonorchis* and *Opisthorchis* eggs. Heterophyid eggs have also been referred to as Opisthorchid-like eggs where the liver fluke is endemic.

Polymerase chain reaction (PCR) may be useful as a sensitive diagnostic tool, particularly for low-intensity *heterophyid* infections.

Treatment

Praziquantel is the drug of choice, given at 25 mg/kg per dose, three doses in 1 day.

Epidemiology

The parasite has been reported in Egypt, Greece, Israel, Western India, Central and South China, Japan, Korea, Taiwan, and the Philippines. Its worldwide distribution may be due to the fact that heterophyids have adapted to snails belonging to various families, and are not very specific with respect to their second intermediate hosts. Both intermediate hosts may be found in different habitats (fresh, brackish, and salt waters), and in different climates. Reservoir hosts include dogs, cats, and birds.

In the Philippines, the prevalence was previously considered low, and its distribution spotty, as shown by previous parasitologic surveys. In the 1980s, less than 1% of 30,000 stools examined in surveys done nationwide were found positive for heterophyid ova. A more recent parasitologic survey done in 1998 in Monkayo, Compostela Valley, however, revealed 31% prevalence with a majority of those infected having moderate to heavy intensities of infection. The species was identified as

Haplorchis taichui. Infection rates were high in both males and females, and in all age groups, especially the working age group. Children and the elderly were not spared of infection. Intestinal heterophyidiasis has since then been recognized as an emerging public health concern in the southern part of the Philippines. Altogether, eight provinces in two regions of Mindanao have reported thousands of cases to date. High prevalence levels were detected in areas where investigations for an outbreak of intestinal capillariasis were being conducted.

Prevention and Control

Preventive measures include avoiding ingestion of raw or improperly cooked fish. It may be difficult to change eating habits. Capacity building of laboratory staff will help in early diagnosis when doing routine stool examination. This will facilitate provision of appropriate treatment. Surveillance in other regions where raw fish (*kinilaw*) is eaten should be considered.

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Liver Flukes

Vicente Y. Belizario, Jr., Raezelle Nadine T. Ciro

Fasciola spp.

These large digenetic trematode species belong to family Fasciolidae. They are parasites found in the liver and biliary passages of humans and herbivorous mammals, especially ruminants. *Fasciola hepatica* (temperate liver fluke) and *F. gigantica* (tropical liver fluke) are the causative agents of fascioliasis. Reports of fascioliasis date back to 1379, and the first detailed descriptions of *F. hepatica* were published in 1523. The presence of *F. hepatica* flukes in humans was first documented in 1760, during an autopsy of a female in Berlin, Germany.

Infections in ruminants result in significant economic loss estimated at 3.2 billion US dollars per annum to rural agricultural communities and commercial producers. In tropical countries, fascioliasis is considered the most important helminth infection of cattle with a reported prevalence ranging from 30 to 90%.

Parasite Biology

The mode of transmission of *F. hepatica* and *F. gigantica* is through the ingestion of metacercariae encysted on edible aquatic plants or by drinking water with viable metacercariae. Upon ingestion, the metacercaria excysts in the duodenum or jejunum, liberating the juvenile fluke, which, in turn, penetrates the intestinal wall to reach the peritoneal cavity where it wanders over the viscera until it penetrates the capsule of Glisson and enters the liver. The parasite then burrows through the liver parenchyma, feeding and growing until it finally enters the bile ducts where it becomes sexually mature in 3 to 4 months (Figure 5.7). The life span of the adult worm is 9 to 13 years.

The adult worm lives in the biliary passages of the liver. Unembryonated eggs are carried by the bile through the sphincter of Oddi into the intestine and subsequently voided with the feces. The eggs mature in water within 9 to 15 days optimally at 15 to 25°C, forming a viable miracidium that escapes through the operculum of the eggshell to seek out and infect the first intermediate host, a snail belonging to family Lymnaeidae. Snail hosts for *F. hepatica* are amphibious which are usually found living on mud. Snail species include *Lymnaea truncatula* (Europe and North Asia), *L. bulmoides* (North America), and *L. tomentosa* (Australia). Snails from family Planorbidae also act as an intermediate host of *F. hepatica* sporadically. On the other hand, the first intermediate hosts for *F. gigantica* are aquatic snails, living in slow-moving bodies of water, which include *L. auricularia* (Asia), *L. acuminata* (Indian Subcontinent), and *L. natalensis* (Africa). In the Philippines, the snail hosts of *Fasciola* spp. are *L. philippinensis* and *L. auricularia rubiginosa*.

Inside the snail, the miracidium develops into a sporocyst, followed by one or two generations of rediae which produce cercariae. Cercariae leave the snail about 5 to 6 weeks after the miracidium entered. After escaping from the snail host, usually at night, the cercaria swims in water, detaches its tail, and encysts in surfaces of aquatic plants forming a metacercaria. The aquatic plants serve as the second intermediate hosts of the parasite. These include *Ipomea obscura* (morning glory or kangkong) and *Nasturtium officinale* (watercress). Cercariae can also encyst freely in water. The metacercaria is the infective stage to the definitive hosts. In the presence of sufficient moisture, the

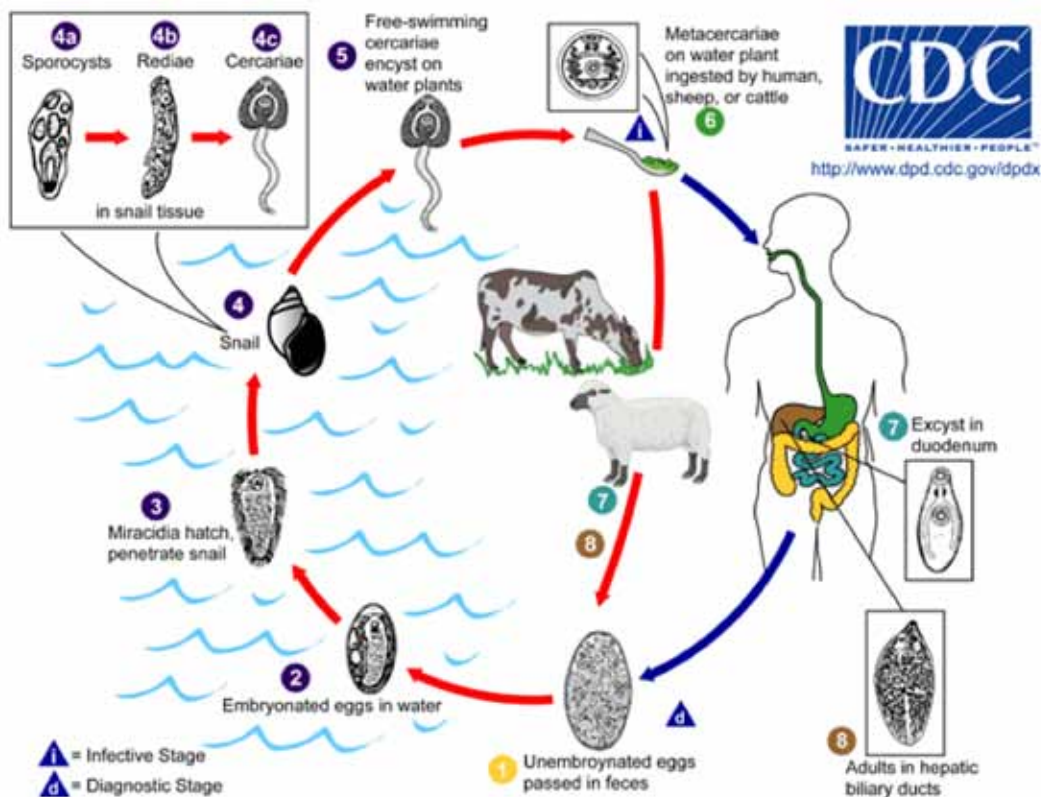


Figure 5.7. Life cycle of *Fasciola* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

metacercariae will remain alive for many weeks, depending on the temperature. They survive longer at a temperature below 20°C; higher temperatures and desiccation will destroy the metacercariae in a short time.

F. hepatica has a large, broad, and flat body which measures 18 to 51 mm in length and 4 to 13 mm in width (near the mid-body). A distinguishing feature is the cephalic cone which has a marked widening at the base of the cone ("shoulder"). The suckers are comparatively small and are located close to each other in the conical projection. The two testes are highly branched occupying the second and third quarters of the body. The ovary is dendritic and situated in front of the anterior testis. The uterus is coiled and relatively short. Vitellaria extend

to the whole lateral field of the hind body. The intestinal ceca are long and highly branched, extending to the posterior end of the body.

Compared to *F. hepatica*, the *F. gigantica* adult worm is longer (25-75 mm), with about the same width (3-12 mm), with less developed shoulders, and a shorter cephalic cone. The ceca are more branched especially towards the midline of the body and the branches of the ovary are longer and more numerous. The average distance between the posterior testes and the posterior border of the body is longer.

The *F. hepatica* egg (Plate 5.18) is large, ovoidal, operculated, and yellowish to brownish in color. It measures 140 to 180 µm by 63 to 90 µm in size and is released from the worm still immature, containing a large unsegmented mass



Plate 5.18. *Fasciola* egg
(Courtesy of the Department of Parasitology,
UP-CPH)

of vitelline cells. The *F. gigantica* egg is slightly larger than the *F. hepatica* egg (160-190 μm by 70-90 μm).

Pathogenesis and Clinical Manifestations

Two clinical stages are recognized in human fascioliasis. An acute stage, which coincides with larval migration and worm maturation in the hepatic tissue, and a chronic stage, which coincides with the persistence of *Fasciola* worms in the biliary ducts.

The acute or invasive phase of human fascioliasis corresponds to the migration of the juvenile parasite from intestine to the liver where it burrows into the liver parenchyma. The damage caused by the parasite penetrating through the intestinal wall and migrating towards the liver is not significant. However, traumatic and necrotic lesions are produced when the parasite burrows through the liver parenchyma. The severity of the injury depends on the number of metacercariae ingested by the host. Though this invasive phase can be asymptomatic, patients have been known to experience dyspepsia, fever, and right upper quadrant abdominal pain. Sudden onset of high fever, hepatomegaly, and marked eosinophilia form a triad of diagnostic significance.

The chronic or latent phase is asymptomatic and corresponds to the period when the parasite has already reached the bile ducts. The adult

worm causes obstruction and stimulates inflammation in the biliary epithelium which subsequently causes fibrosis. The thickened fibrous ducts, in turn, cause less bile to be passed out building up back pressure. In heavy infections, atrophy of the liver parenchyma and concomitant periductal cirrhosis ensue. The wall of the bile duct may be eroded allowing the worms to re-enter the liver parenchyma and cause large abscesses to develop. Other complications include obstructive jaundice, hemobilia, and biliary cirrhosis. Associated lithiasis of the bile ducts or gallbladder is also common, as the eggs or fragments of dead parasites can form nuclei for calculi. Another rare complication of fascioliasis is acute pancreatitis. In some cases, this phase is only diagnosed during a surgery.

During the migration from the intestine to the liver, the parasite may wander or be carried hematogenously (after it had penetrated a blood vessel) to ectopic sites such as the lungs, subcutaneous tissue, the brain, and the orbit where abscesses or fibrotic lesions may also result.

Another unusual form of fascioliasis can occur after ingestion of raw *Fasciola*-infected liver. Flukes surviving mastication attach to the posterior pharynx, causing hemorrhagic nasopharyngitis and dysphagia, known as *halzoun* in Lebanon and *marrara* in Sudan.

Diagnosis

In majority of cases, diagnosis of the infection, whether in the acute or chronic phase is difficult because of overlapping symptoms, or because of lack of symptoms. This is compounded by the intermittent passage of eggs by the adult worm. Determining the phase of infection will therefore depend on the clinical suspicion. A history of eating raw, improperly cooked freshwater vegetation or of living in or travel to an endemic area is suggestive of infection. Selection of adequate serological and coprological methods can help determine the

phase of infection when applied to the acute or chronic stages, respectively.

Differentials for human fascioliasis include diseases which may present with similar symptoms such as acute viral hepatitis, schistosomiasis, visceral toxocariasis, biliary tract diseases, and hepatic amebiasis.

Parasitological diagnosis may be made through the identification of eggs in stool, duodenal contents, or bile, or the recovery of adult worms during surgical exploration, after treatment, or at autopsy. However, the eggs may be present in very small numbers at irregular intervals and thus may be difficult to find. Eggs may also be transiently present in the stool after ingestion of poorly cooked liver from infected animals (spurious or false fascioliasis). This situation, with its potential for misdiagnosis, can be avoided by having the patient follow a liver-free diet several days before a repeat stool examination.

Although techniques for showing the presence of eggs in stools have long been used to confirm the diagnosis, these methods have limitations in determining human fascioliasis because parasite eggs are not found in feces until three to four months after infection, and due to low sensitivity in low-intensity infections. Because the release of *Fasciola* coproantigens takes place before egg shedding, immunologic methods are preferable to egg examination for the detection of acute infections. Immunodiagnosis including enzyme-linked immunosorbent assay (ELISA) and Western blot are now widely applied as alternative methods of confirming early and extrabiliary human fascioliasis.

Radiological examinations may also help in the diagnosis of fascioliasis. Radiological findings of fascioliasis, mainly on sonography and computed tomography (CT), have been described in several reports. In the hepatic phase of the disease, parenchymal lesions are due to migration of the parasites through the liver. The characteristic features on CT are described as multiple confluent, hypodense

nodules and tunnel-like branching hypodense tracts. Hepatic sonographic findings have been described as small clustered hypoechoic lesions with poorly defined contours and hypoechoic nodular lesions. The biliary phase of the disease occurs in the presence of parasites in the biliary system. Sonography is the useful method in the detection of biliary lesions. The oval shaped, leaf-like, or snail-like echogenic structures with no acoustic shadowing in the gall bladder or common bile duct have been described as characteristics of fascioliasis. Endoscopic retrograde cholangiopancreatography (ERCP) can also be used in diagnosing fascioliasis in the biliary phase, since it can demonstrate biliary obstruction or filling defects.

Treatment

Triclabendazole is the drug of choice for treating fascioliasis because of its efficacy, safety, and ease of use. The first report of successful treatment of human fascioliasis with triclabendazole dates back to 1986. The recommended treatment is a single 10 mg/kg oral dose of triclabendazole following food intake. For individuals with heavy infections, the recommended treatment is two doses of 10 mg/kg spaced by 12 hours. Mild and transient abdominal pain, biliary colic, fever, nausea, pruritus, vomiting, weakness, liver enlargement, and mild, limited disturbances in liver function have been observed as adverse events associated with the drug. Liver flukes resistant to triclabendazole have been found in livestock, probably due to the widespread use of the drug. Resistant *F. hepatica* have been reported in Australia, Ireland, the Netherlands, Scotland, and recently, in Spain. No resistance in *Fasciola* infecting humans has been reported so far.

Bithionol may also be used to treat fascioliasis. The fasciolicidal activity of bithionol was first described in the early 1960s. Cure rates ranging from 58 to 100% have been reported. Although bithionol is no longer

commercially available for human use in many countries, it is still used for the treatment of fascioliasis (e.g., in the United States by the Centers for Disease Control and Prevention) because the drug is often more readily available than triclabendazole. Adverse events including anorexia, nausea, vomiting, and abdominal pain are mild and transient. A key drawback of bithionol is that long treatment duration is necessary. Bithionol is given at 30 to 50 mg/kg body weight on alternate days to complete 10 to 15 doses.

Peroxidic compounds, such as semi-synthetic artemisinins and synthetic trioxolanes, which are known for their antimalarial and antischistosomal properties, have been reported to show trematocidal activities. Single 200 to 400 mg/kg oral doses of artesunate and artemether completely cured chronic *F. hepatica* infections in rats.

Epidemiology

Fascioliasis has a worldwide distribution and is of great economic importance in livestock-raising countries. The prevalence in animals in Central and Latin America is about 25% but may reach 70% in cattle, goats, and sheep in other countries. In the Philippines, the dominant species affecting cattle and water buffaloes is *F. gigantica*. Examination of cows, carabaos, and horses in South Cotabato in 2007 showed a fascioliasis prevalence of 89.5%. Human fascioliasis is typically sporadic. However, clinical cases and some outbreaks have recently occurred. The estimated number of people with fascioliasis is 360,000 in Bolivia, 830,000 in Ecuador, 10,000 in Islamic Republic of Iran, 742,000 in Peru, and 37,000 in Yemen. The total estimated number of people infected is 2.4 to 17 million, in 51 countries, from five continents. The number of persons at risk is more than 180 million worldwide.

Fascioliasis due to *F. gigantica* is typical of rural areas of Vietnam, but is not infrequent in areas around urban centers as well. About 5,000

patients are estimated to require treatment each year. There has been an increase in the number of cases reported, in response to the availability of treatment. Transmission to humans is highly linked to eating raw water-grown vegetables that harbor *F. gigantica* metacercariae. Washing vegetables with water, vinegar, or lemon juice is not sufficient to remove the encysted metacercariae. Use of contaminated kitchen tools in preparing other foods can also cause the metacercariae to be transmitted.

In Asia, most human cases have been reported in Iran, especially in Gilan Province, on the Caspian Sea. In parts of eastern Asia, human fascioliasis appears to be sporadic. Few cases have been documented in Japan, Korean peninsula, and Thailand. In the Philippines, no case of human fascioliasis has been documented.

In Europe, human fascioliasis mainly occurs in France, Spain, Portugal, and the former USSR. France is considered an important human endemic area. A total of 5,863 cases have been recorded from nine French hospitals from 1970 to 1982.

Prevention and Control

Preventive measures include thorough washing or cooking of vegetables, and boiling of water in areas where the infection is endemic. Cilla et al. in 2001 reported the decrease in infection over the years in Gipuzkoa, Spain which is probably related to a change in dietary habits. Control measures include elimination of the snail intermediate host through the application of copper sulfate, and killing the parasite in the reservoir host by chemotherapy.

Spitfill and Dalton in 1998 demonstrated that animals can be significantly protected against infection by vaccination with defined *Fasciola* antigens. These include a fatty-acid binding protein (FABP) termed Fh12, glutathione-S-transferase (GST), cathepsin L (CatL) proteinase, and hemoglobin (Hb). Apart from reducing fluke burden, some vaccines have elicited concurrent reductions

in parasite egg production. It was also noted that in those vaccinated with cathepsin L2-Hb, >98% of the eggs recovered did not embryonate to miracidia. A juvenile protease known as *F. hepatica* cathepsin B2 (FhCB2) was also recently validated as a vaccine for fascioliasis using the rat model. The FhCB2 vaccine was shown to be highly immunogenic, induced a 60% reduction in fluke burden, and a 63% reduction in the size of the recovered flukes. Vaccination with FhCB2 also led to significantly reduced liver damage (61%), suggesting a killing effect on young parasites before extensive damage occurs in the liver. A commercially feasible vaccine that might also reduce parasite transmission and reduce the chances of liver damage in the field is a realistic goal. Alternative adjuvants, routes of delivery, as well as the production of a recombinant protein that mimics the protection of the native protein are among the latest developments.

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Clonorchis sinensis

Opisthorchis felineus
Opisthorchis viverrini

These small digenetic trematodes belong to family Opisthorchiidae, and are parasites of the bile duct and gallbladder of humans and fish-eating mammals. Infections of humans by *Opisthorchis felineus* and *O. viverrini* were first recorded in 1892 and 1911, respectively. The liver fluke *Clonorchis sinensis* was first reported in India in 1874, during an autopsy of a 20-year old Chinese patient.

Parasite Biology

The liver flukes, *C. sinensis*, *O. felineus*, and *O. viverrini*, have similar life cycles (Figures 5.8–5.9). The usual mode of transmission is via ingestion of the metacercaria of the parasite present in infected raw or undercooked fish. Viable encysted metacercariae have been reported in salted, dried, or pickled fresh water fish. Metacercariae from decomposing fish could potentially be ingested by drinking contaminated water.

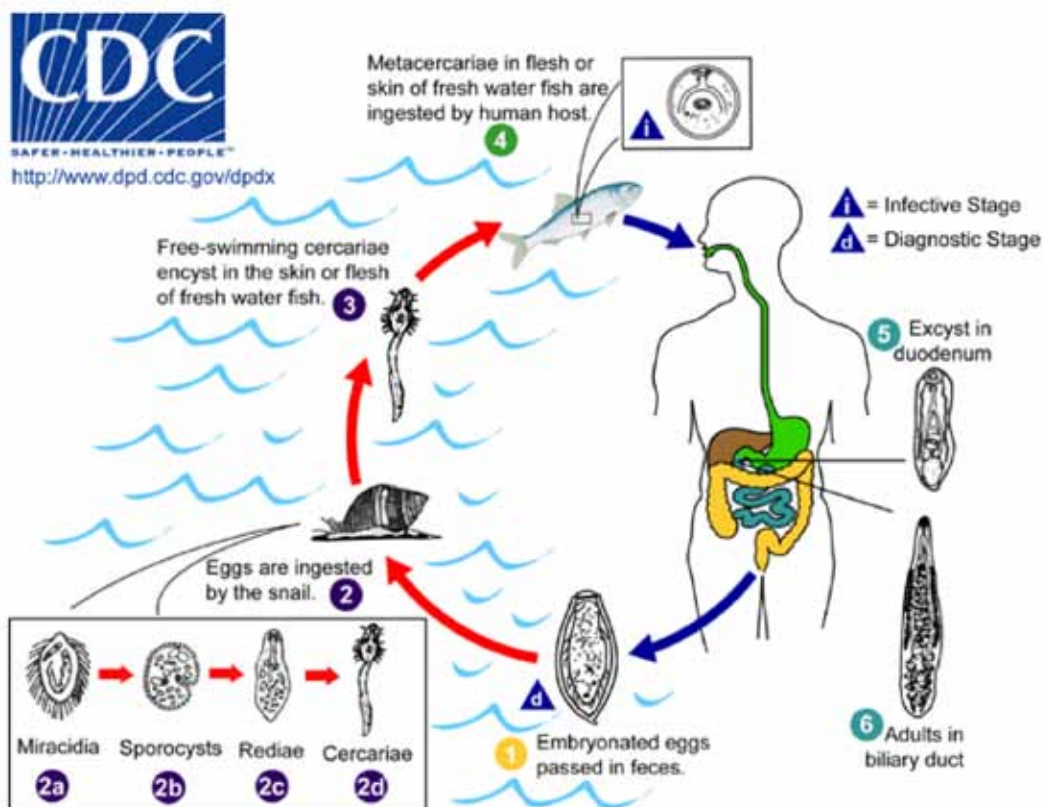


Figure 5.8. Life cycle of *Clonorchis sinensis*
 (Accessed from www.dpd.cdc.gov/dpdx)

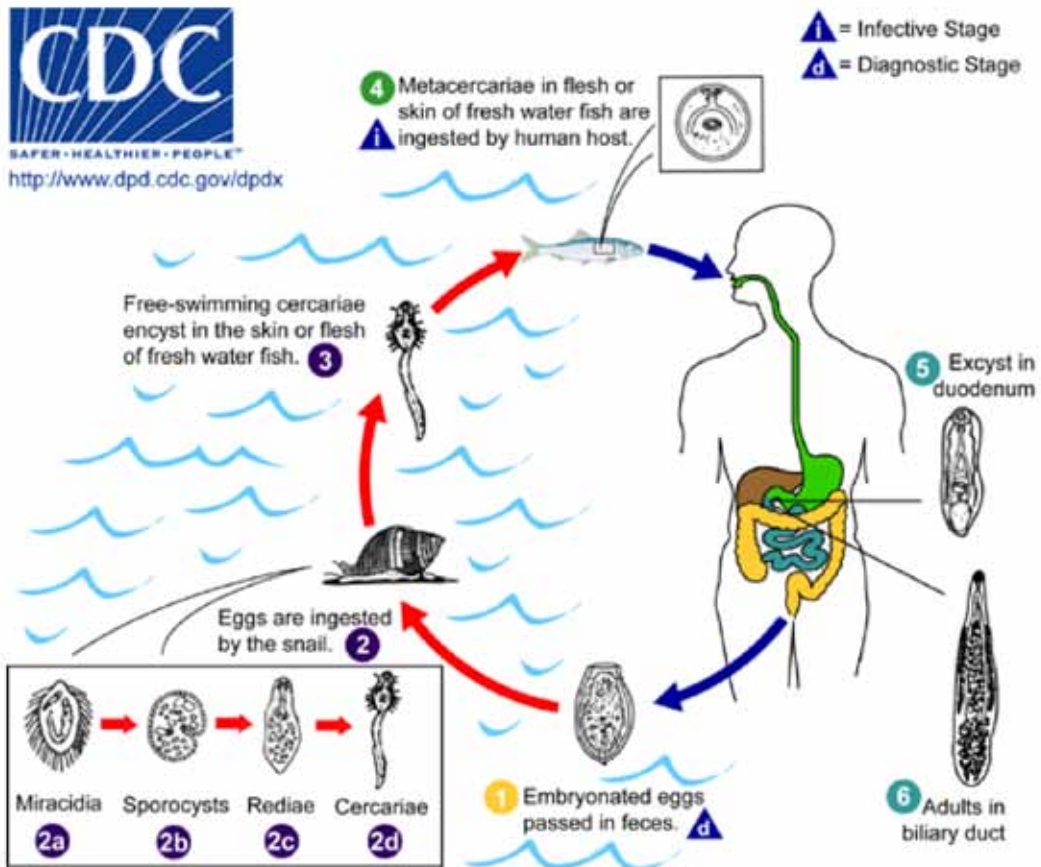


Figure 5.9. Life cycle of *Opisthorchis* spp.
 (Accessed from www.dpd.cdc.gov/dpdx)

The metacercaria excysts in the duodenum, and the young fluke moves through the ampulla of Vater to the common bile duct, and then to the distal biliary capillaries where it matures into an adult worm. The adult fluke attaches itself to the mucosa of the bile duct by using its suckers, and by embedding itself in sticky mucus without causing permanent ulceration of the epithelial lining. The flukes may also be found in the pancreatic duct and the gallbladder. The worm feeds on tissue fluids, red blood cells, and mucus.

The egg is fully mature when it is released from the worm. It passes with the bile to the intestine, and escapes into the environment with

the feces. The miracidium hatches only after the egg is ingested by the first intermediate host. The first snail intermediate host of *C. sinensis* belongs to the following genera: *Parafossarulus* (*P. manchouricus*, *P. anomalospiralis*, and *P. stratulus*), *Bulinus* (*B. striatulus*), *Semisulcospira*, *Alocinma* (*A. longicornis*), *Thiara* (*T. granifera*), and *Melanoides* (*M. tuberculatus*). On the other hand, *O. felineus* and *O. viverrini* require snails belonging to the genus *Bithynia*.

Upon entry into the snail host, the miracidium transforms into a sporocyst, which subsequently produces rediae. Each redia, in turn, produces cercariae that are released into the surrounding water. Upon contact with the

second intermediate host, a fresh water fish, the cercaria attaches itself to the host epithelium with its suckers, and encysts as metacercaria under a scale or in a muscle.

There are many fish species that serve as intermediate hosts of these parasites, but the majority belongs to family Cyprinidae. A total of 31 species in seven families of freshwater fish, and one species of freshwater shrimp, have been recorded as second intermediate hosts of *C. sinensis*. Metacercariae of *Opisthorchis* spp. have been recorded in 23 species and 2 subspecies of Cyprinidae family, and 11 species of Cobitidae family.

The metacercaria is the infective stage to the definitive host. One study in northeast Thailand showed that seasonal variations in metacercariae was a common phenomenon in areas with both high and low endemic infection. The metacercarial load in fish was shown to be positively associated with infection levels among humans.

Adult worms are also found in the bile ducts of cats, dogs, pigs, and six other species of mammals, which can act as reservoir hosts. Adults of the three parasites are leaf-like in shape, with transparent tegument. The *C. sinensis* adult is 10 to 25 mm long and 3 to 5 mm wide, while *Opisthorchis* adults are slightly shorter, being 8 to 12 mm long and 1.5 to 3 mm wide. The main similarity between *C. sinensis* and *Opisthorchis* spp. is the location of the vitellaria, which are found in the middle third of the body at the level of the uterus; whereas the main differences are in the morphology and arrangement of their testes. *C. sinensis* adults have two large, highly branched testes arranged in tandem in the posterior half of the body. *Opisthorchis* adults, however, have lobate testes, which are arranged obliquely. The *O. viverrini* adult can be differentiated from the *O. felineus* adult on the basis of testes morphology. The testes of *O. viverrini*, which are positioned close to each other, are more deeply lobulated (Plate 5.19).

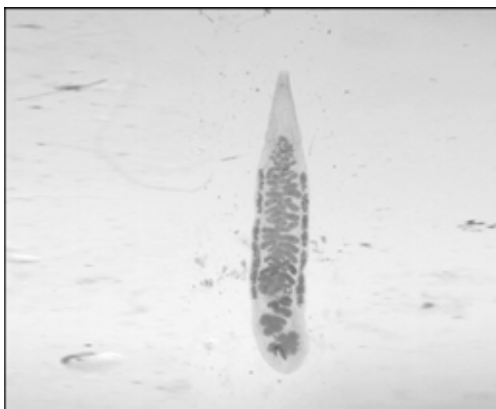


Plate 5.19. *Opisthorchis viverrini* adult
(Courtesy of the Department of Parasitology,
UP-CPH)

Eggs of these parasites are yellowish brown, ovoid, and measure 26 to 30 μm by 15 to 17 μm . There is a distinctly convex operculum that fits into the thickened rim of the eggshell, and a small protuberance at the abopercular end. Inside the egg is a well-developed miracidium that has asymmetrical features. Eggs of the three species of liver flukes are difficult to differentiate.

Pathogenesis and Clinical Manifestations

In clonorchiasis, metacercariae reaching the biliary system mature and provoke pathological changes as a result of local trauma and irritation. Although the morphologic features vary with duration and severity of the infection, they are sufficiently distinctive and characteristic to allow classification into phases. These phases are as follows: (a) desquamation of epithelial cells; (b) hyperplasia and desquamation of epithelial cells; (c) hyperplasia, desquamation of epithelial cells, and adenomatous tissue formation; and (d) marked proliferation of the periductal connective tissue with scattered abortive acini of epithelial cells, and fibrosis of the wall of the biliary duct.

In general, light infections with *C. sinensis* (≤ 100 flukes) are asymptomatic, or have few

non-specific clinical signs, such as diarrhea and abdominal pain. Infections with a moderate parasite load (101-1,000 flukes) may cause fever, diarrhea, loss of appetite, rash, edema, night blindness, swollen abdomen, and enlargement of the liver. Patients with a very high worm burden (up to 25,000 flukes) may also present with acute pain in the right upper quadrant. Often, the acute symptoms subside after a few weeks, and are followed by chronic complications. In the chronic stages, liver malfunction can occur. Calculi, acute suppurative cholangitis, recurrent pyogenic cholangitis, cholecystitis, hepatitis, and pancreatitis are among the more severe late complications.

An increased risk of developing hepatocellular carcinoma and cholangiocarcinoma are among the most significant sequelae. *C. sinensis* has been classified by the International Agency for Research on Cancer (IARC) as a probable carcinogen (group 2A).

Infections with *O. viverrini* are often asymptomatic, particularly those of light intensity. Flatulence, fatigue, dyspepsia, right upper quadrant abdominal pain, anorexia, and mild hepatomegaly occur in approximately 5 to 10% of infections. Severe infections, which are rare, might cause obstructive jaundice, cirrhosis, cholangitis, acalculous cholecystitis, or bile peritonitis.

Cholangiocarcinoma is the most serious complication of infection with *O. viverrini*. Studies carried out in the northeastern part of Thailand found a positive correlation between the endemicity of opisthorchiasis and the frequency of cholangiocarcinoma. Although the pathophysiology is not entirely understood, many factors are likely involved in carcinogenesis, including mechanical and chemical irritation of the tissue by the flukes, and host immune responses.

In contrast to infections with *C. sinensis* and *O. viverrini*, many patients infected with *O. felinus* suffer from fever and hepatitis-like symptoms in the acute stage of infection. Right

upper quadrant abdominal pain, nausea, and emesis have been reported. Chronic symptoms include biliary tract obstruction, inflammation, and fibrosis, as well as liver abscess formation, pancreatitis, and suppurative cholangitis.

A. Correlation of Opisthorchiasis and Clonorchiasis with Cholangiocarcinoma

Opisthorchis and *Clonorchis* parasitize the bile ducts of millions of individuals in the Far East. The most important aspect of infection with these flukes is their role in carcinogenesis. Numerous studies have shown that these flukes are closely associated with the development of cholangiocarcinoma. The link between *C. sinensis* and cholangiocarcinoma is supported by epidemiological data. In 1956, it was estimated that 15% of primary liver cancers in Hong Kong were cholangiocarcinomas associated with *C. sinensis*. A study of 2,635 necropsy cases in Thailand showed that 78% of cholangiocarcinomas were associated with liver fluke infection. In certain areas of Korea with an extremely high prevalence of *Clonorchis*, fluke infection increased the relative risk of cholangiocarcinoma six-fold. Experimental studies in animals have confirmed the carcinogenic potential of these parasites.

Studies carried out in the northeastern part of Thailand found a positive correlation between the endemicity of opisthorchiasis and the frequency of cholangiocarcinoma. The highest incidence of cholangiocarcinoma has been reported for areas where *O. viverrini* is highly endemic. Sakol Nakhon (upper Northeast Thailand) has the highest national mortality rate of liver and bile duct cancer, at 61.4 attributed deaths per 100,000 people. A similar association between opisthorchiasis and bile duct cancer has been observed in Lao PDR, where the prevalence of *O. viverrini* is high.

The pathogenesis of *Clonorchis* and *Opisthorchis*-associated cholangiocarcinoma involves several mechanisms. Chronic irritation and inflammation caused by the fluke can result

in hyperplasia and adenomatous changes of the biliary epithelium. Hyperplastic cells are vulnerable to carcinogens that can easily induce DNA damage during active cell proliferation. Liver fluke infection results in endogenous formation of N-nitroso compounds in the area around the bile ducts, which in turn may lead to neoplastic transformation. Furthermore, macrophages and other inflammatory cells, activated by parasite-specific T-cells, synthesize nitric oxide, which is a potential carcinogen. It is likely that several of the above mechanisms are involved in the carcinogenesis process.

Mucin-producing activity is also a frequent feature reflecting the neoplastic transformation of goblet cells in the bile duct lining. Application of various carcinogens to liver fluke-infected animals has been shown to increase the incidence of cholangiocarcinoma.

Diagnosis

Diagnosis is by detection of the parasite egg in the stool. *Clonorchis*, *Opisthorchis*, and heterophyid eggs are difficult to differentiate under an ordinary light microscope. Eggs, when stained with potassium permanganate and examined under 400x magnification, show distinct melon-like ridges on the surface of *O. viverrini* eggs, while there is a light striae pattern on *Haplorchis taichui* (heterophyid) eggs.

Cholangiography is a very useful diagnostic tool. Several radiological features of biliary clonorchiasis have been described, including saccular dilations of the intrahepatic bile ducts, and rapid ductal tapering toward the periphery (referred to as the "arrowhead sign"). Less dramatic ductal wall irregularities may also be seen, such as indentations, a scalloped appearance, and, occasionally, linear or elliptical filling defects representing free-floating worms.

ELISA with crude extracts of adult *C. sinensis* has been reported to have a high degree of sensitivity and a moderate degree of specificity for the serodiagnosis of clonorchiasis. Enzyme immunoassay (EIA)

and coproovoscopy are concurrently used to define the spread of clonorchiasis in certain regions in Russia. It shows the efficiency of EIA in seroepidemiological surveys and the possibility of its use in endemic areas. The assay is recommended for wide application in clinical and epidemiological practice in the foci of the disease.

A polymerase chain reaction (PCR) method developed with 100% sensitivity has been used for detecting a single *O. viverrini* egg in artificially inoculated feces. The method is useful for specific identification of *O. viverrini* eggs in stool samples without the risk of false positives. A single, one-step multiplex PCR, targeting mitochondrial DNA, permits the detection and discrimination of *Clonorchis sinensis* and *Opisthorchis viverrini* in different life-stage forms, from fish intermediate hosts, and from infected patients. This multiplex PCR technique produced no cross reaction between *C. sinensis* and *O. viverrini*, or with metacercariae of other trematodes commonly found in fish, or eggs from mixed infections in humans.

Treatment

Praziquantel is given at 25 mg/kg, three times a day for 2 days. It may also be given at 60 mg/kg in three doses for 1 day. The latter regimen has been found to have a 96% cure rate and 99% egg reduction rate.

The therapeutic effect of albendazole is comparable to praziquantel. It has the advantage of clearing various intestinal helminthiasis simultaneously, with very low toxicity, excellent tolerance, and relatively low cost. However, the seven-day treatment course is longer than the course for praziquantel.

A study has shown that in cases of light to moderate infection, a praziquantel-albendazole combination is more effective than praziquantel alone. The combination was also found to be highly effective for treating cases of co-infection with *Ascaris*, *Trichuris*, and hookworm.

Agents and biologically active fractions derived from medicinal plants grown in Siberia have been tested *in vitro* and *in vivo*. The extract from the aspen bark displayed the highest activity against *Opisthorchis*. The results of chemical and chromatographic studies have indicated that active fractions contain salicin and its derivatives. The aspen bark produces no substantial toxic effect in laboratory animals and belongs to the class “low toxic substances.”

The artemisinins and synthetic peroxides (i.e., OZ78) also possess trematocidal properties against schistosomes, *C. sinensis*, and *Fasciola hepatica in vivo*. Tribendimidine also shows activity against the intestinal trematode *Echinostoma caproni*, *C. sinensis*, and *O. viverrini*. A single 150 mg/kg of body weight oral dose of either artemether, artesunate, or tribendimidine resulted in worm burden reductions of 99 to 100% in rats harboring adult *C. sinensis*. OZ78, at a single 300 mg/kg oral dose, achieved a worm burden reduction of 98.5% against adult *C. sinensis* in rats.

Epidemiology

Transmission of clonorchiasis and opisthorchiasis is by consumption of raw, undercooked, salted, dried, or pickled freshwater fish that harbor encysted metacercariae. Reservoir hosts are fish-eating mammals such as dogs, cats, and rats.

Current global estimates for *C. sinensis* infection is 35 million, with 601 million people at-risk of acquiring the infection. The estimated number of persons infected with *O. viverrini* is 9 million, with 68 million people at-risk, while about 1.2 million are estimated to be infected with *O. felineus*, and 12.5 million at-risk.

O. viverrini and *C. sinensis* chronically infect over 30 million people in Southeast Asia, resulting in significant morbidity and predisposition to cholangiocarcinoma. *C. sinensis* is endemic in China, Korea, Japan, and Vietnam; *O. felineus* has been reported in Europe, Turkey, the former USSR countries,

Korea, Japan, Vietnam, and India; and *O. viverrini* in Thailand, Laos, Malaysia, and in immigrants to North America. A case of a Chinese immigrant with clonorchiasis in Australia has been reported. The patient was said to have harbored the parasite for 26 years without developing neoplasia. A case of opisthorchiasis has been reported from the Davao Medical Center in the Philippines. The parasite was recovered during a surgical operation of the bile ducts.

O. viverrini infections remain a major public health problem in Northeast Thailand, where approximately one-third of the population is infected. The northeast region is largely populated by Thais and people of Laotian descent who eat raw fish, which harbor the infective stage of the fluke.

The distribution of liver fluke disease is related, in part, to the distribution of intermediate hosts and animal reservoir hosts. Traditional consumption of improperly cooked fish, and indiscriminate defecation habits among rural inhabitants are significant factors that determine the high prevalence of liver fluke infection in an area.

Prevention and Control

The main strategies for liver fluke control consist of three interrelated approaches, namely: (a) stool examination and treatment of positive cases with praziquantel in order to eliminate human host reservoir, (b) health education for the promotion of cooked fish consumption in order to prevent infection, and (c) proper human waste disposal in order to interrupt transmission.

An alternative approach to control transmission is by making the fish intermediate host safe for consumption. A study suggested that irradiating fish at a dose of 0.15 kGy could control the infectivity of *C. sinensis* metacercariae. Freezing or storing infected freshwater fish in heavy salt may not be effective in the prevention of clonorchiasis. Acetic acid

(3-6%) pretreatment for four hours increases the salt penetration rate into the muscles of fish, which accelerates the death of *O. felineus* metacercariae.

In the Philippines, only two cases of clonorchiasis, both in foreigners and likely imported, had been diagnosed at the College of Public Health, University of the Philippines Manila.

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Arthropods and Mollusks of Medical Importance

Introduction to Arthropods of Medical Importance

Lillian A. de las Llagas

Of all the major divisions or phyla which make up the animal kingdom, Phylum Arthropoda is certainly one of the most important. Eighty-five percent of all known animals are arthropods. These are bilaterally symmetrical invertebrate animals with segmented bodies, jointed appendages, and hard outer coverings or exoskeletons. No other animal group demonstrates such a great diversity in structure, life cycle, and habits. The arthropods range in size from the Atlas moth with a wingspread of 12 inches, to the small follicle mite, less than 1/250 of an inch long.

Some arthropods are parasitic, while most are non-parasitic. Some prefer to live in highly organized and complex environments in which each member contributes something to others in a symbiotic relationship.

Many arthropods have complicated life histories. In some, the entire life cycle is not completely known. Some demonstrate little change in morphology throughout the different life stages, while others pass through a complete metamorphosis having egg, larval, pupal, and adult stages.

Metamorphosis refers to the change in form or structure of an arthropod that occurs during the period of development. A few primitive insects develop without metamorphosis. In these insects, the young is the exact replica of the adult differing only in size.

There are two types of metamorphoses:

- *Gradual or incomplete metamorphosis.* In this type of metamorphosis, an arthropod undergoes three stages: egg, nymph, and adult. The young resembles the adult except for the smaller size and sexual immaturity. Examples of arthropods exhibiting this include cockroaches, grasshoppers, lice, and bugs.
- *Complete metamorphosis.* In this type of metamorphosis, an arthropod undergoes four stages: egg, larva, pupa, and adult. There is a great difference between the immature stages and the adults. Examples of these are mosquitoes, flies, butterflies, moths, ants, bees, wasps, fleas, and beetles.

Arthropods are found everywhere, whether it is in mountains, swamps, deserts, cities, or countryside. Their presence in any environment reflects their capability to adapt, propagate, and establish colonies.

Arthropods are provided with special mechanisms which they use against their enemies: the chitinized exoskeleton, primarily a nitrogenous polysaccharide which makes the integument impervious to water; appendages which may be lost and later regenerated; hairs, scales or spines; and body fluids which may be used effectively for their survival.

Classification of Arthropods

Phylum Arthropoda comprises at least 740,000 species. The majority of medically important arthropods can be grouped into two classes: Insecta and Arachnida. Other classes, which are also important, are Chilopoda, Diplopoda, Crustacea, and Pentastomida (Table 6.1).

Table 6.1 . List of immediate diagnostic features of arthropods

Class	Antennae	Legs
I. Crustacea (crabs, lobster, shrimps, copepods)	2 pairs	5 or more pairs of walking legs
II. Arachnida (mites, ticks, scorpions, spiders)	0	4 pairs (adult stage)
III. Hexapoda/Insecta (mosquitoes, flies, lice, bugs, etc.)	1 pair	3 pairs (adult stage)
IV. Chilopoda (centipedes)	1 pair	1 pair per body segment
V. Diplopoda (millipedes)	1 pair	2 pairs per body segment
VI. Pentastomida (tongue worms)	0	0

Class Insecta (flies, mosquitoes, bees, wasps, butterflies, bugs, etc.) is considered the largest, representing approximately 70% of the phylum. It also typifies the arthropod’s external and internal structures. Class Insecta is the most important group of arthropods from the medical viewpoint. It includes many species that directly and indirectly affect humans.

External Anatomy

The body of an insect is divided into three major regions: the head, thorax, and abdomen (Figure 6.1). In many insects, these parts are clearly well-differentiated, as in flies and mosquitoes, whereas in some, they are less distinct as in fleas.

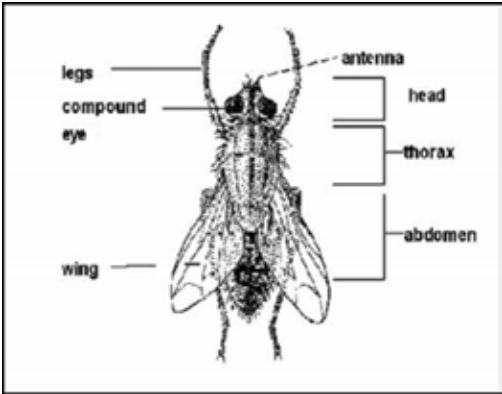


Figure 6.1 . A generalized diagram of an adult Cyclorraphan fly (From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

A. Head

The head bears the eyes, antennae, and the mouthparts. The antennae are located in the front portion of the head between the eyes. They are greatly modified, often having characteristic shapes, and are provided with chemoreceptors (Figure 6.2).

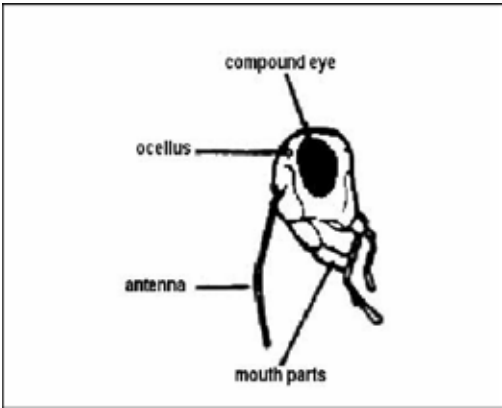


Figure 6.2. Parts of an insect head (From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

Two types of eyes occur in insects: simple and compound. Simple eyes or ocelli consist of single eye units or facets. Compound eyes are usually very large and maybe round, oval, or

kidney-shaped. The outer face of the compound eye is composed of many small six-sided lenses called facets. In general, the active flying insects have large eyes with many facets, while the walking types have fewer facets. Some parasitic insects have poorly developed eyes, as in some fleas.

Insects have an upper lip or labrum, a lower lip or labium, a pair of maxillae or upper jaw, and a pair of mandibles or lower jaw. The shapes and sizes of these structures vary according to the insects' feeding habits. There are four principal types of mouthparts:

1. Chewing mouthparts

These are exemplified by cockroaches and silverfish, which use their mouthparts to grind solid food. The mandibles are useful in cutting or tearing food apart. The maxillae, labrum, and labium are used in handling food before it is swallowed. The palpi are used to feel, smell, and taste food. These appendages are provided with hairs where the various senses are concentrated (Figure 6.3).

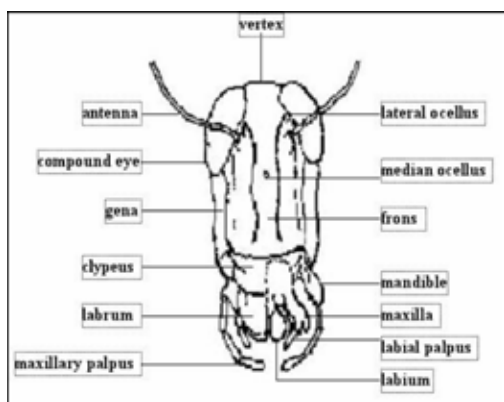


Figure 6.3. Chewing type of mouthparts (From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

2. Sponging mouthparts

This type, as exemplified by houseflies, is adapted for sucking up liquid or readily

soluble foods. The mandibles are absent, and the maxillae are represented only by the palps. The labrum and labium fuse to form a proboscis with a spongy tip called the labellum. The insect regurgitates saliva to dissolve the food. Then, the capillary grooves at the base of the labellum carry the liquefied food to the food canal inside the proboscis (Figure 6.4).

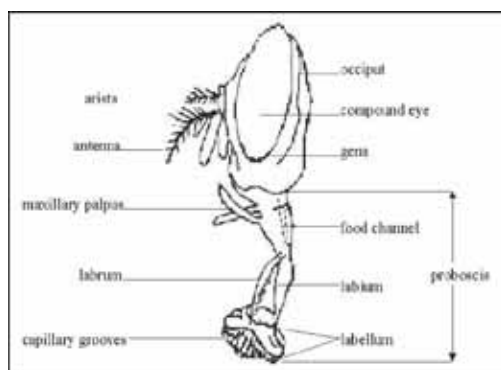


Figure 6.4. Sponging type of mouthparts (From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

3. Piercing-sucking mouthparts

These are exemplified by mosquitoes, biting flies, sucking lice, fleas, and kissing bugs. The mandibles, labrum, and maxillae are long and slender. The labium forms a stout sheath, which holds these structures, and the entire structure is called the proboscis (Figure 6.5).

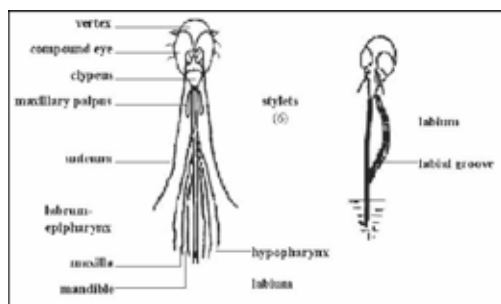


Figure 6.5. Piercing-sucking type of mouthparts (From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

4. *Chewing-lapping mouthparts*

An example of an insect having this type of mouthparts is the honeybee. Mandibles and maxillae are of the chewing type and are used for grasping prey or for molding wax or nest material (Figure 6.6).

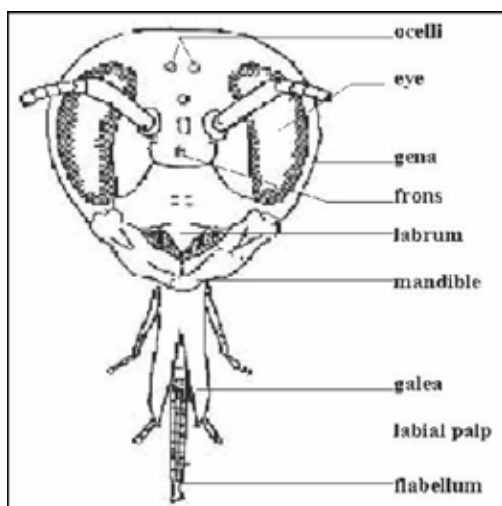


Figure 6.6. Chewing-lapping type of mouthparts (From Baltazar CR, Salazar NP. *Philippine insects: an introduction*. Quezon City: University of the Philippines Press; 1979.)

B. *Thorax*

This is the second main body region which is connected to the head by a membranous region, called the neck or cervix. This part bears three segments, namely: prothorax, mesothorax, and metathorax. Each segment bears a pair of walking legs. Wings, when present, are attached to the mesothorax and metathorax.

1. *Wings*

These are membranous extensions of the body wall and consist of an upper and lower layer. These layers are supported by reinforcing structures, which appear as distinct lines called veins.

Wing veins running from the base to the apex of the wings are called longitudinal veins. Cross veins connect the longitudinal veins. The arrangement and number of these veins are important in the classification of insects. Areas in between veins are called cells. Some veins may be closed. Each vein contains a nerve cord, trachea, and hemolymph. The leading edge is called the costa, and short subcostal veins are numbered 1, 2, 3 and so on.

2. *Leg*

The leg is divided into the coxa, trochanter, femur, tibia, tarsus, and pretarsus (Figure 6.7). The femur and tibia correspond to the human thigh and shin, and the tarsus has a function similar to that of the foot. The last tarsal segment usually terminates into a pair of claws or pulvilli, which help the insects in walking on smooth surfaces.

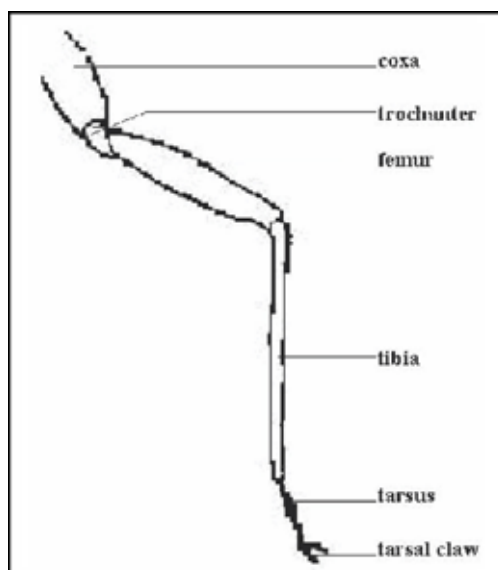


Figure 6.7. Walking leg of an insect (From Baltazar CR, Salazar NP. *Philippine insects: an introduction*. Quezon City: University of the Philippines Press; 1979.)

C. Abdomen

The third body region, which bears the spiracles and the external reproductive organs, is made up of 11 segments. The spiracles (Figure 6.8) are the external openings of the respiratory system, and some insects have a pair on each abdominal segment. The 8th and 9th segments bear the external sex organs used for copulation in the male and serve as an egg-laying device or ovipositor for the female. Some bear a pair of finger-like processes called cerci (Figure 6.9) on the 11th segment which are more conspicuous in females.

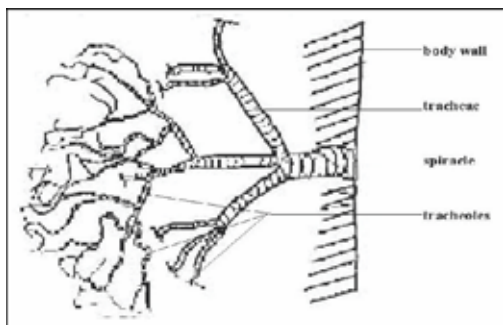


Figure 6.8. Spiracle
(Courtesy of Dr. Lilian de las Llagas)

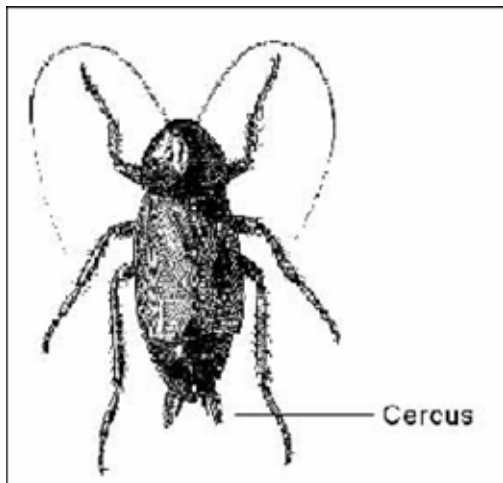


Figure 6.9. Cercus
(From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

Internal Anatomy

A. Circulatory System

Insect blood is usually colorless and is called hemolymph. It contains hemocytes, which are blood cells that are mainly phagocytic. Blood circulation is maintained by the hemolymph which flows through small valve-like openings called ostia (Figure 6.10). The heart is located dorsally and blood from the heart is forced forward through the aorta to the brain. The main function of the heart is to carry nutrients to the tissues, and waste products to the Malpighian tubules for excretion (excretory organ). The entire body cavity is called the hemocoel.

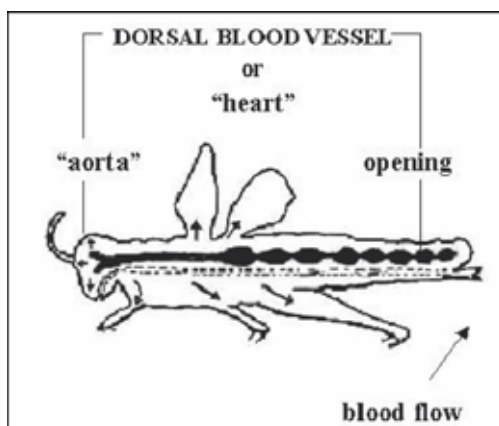


Figure 6.10. Diagram of an insect showing the arrangement of the circulatory system
(From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

B. Respiratory System

Oxygen reaches the tissues by direct gaseous exchange. The spiracles, which are circular openings in the cuticle, allow air to enter the body (Figure 6.11). Spiracles are located on the mesothorax, the metathorax, and the first eight abdominal segments. Air passes through a small tube called the trachea. Oxygen diffuses across the tracheoles into the cells, while carbon dioxide from the cells enters the tracheoles and

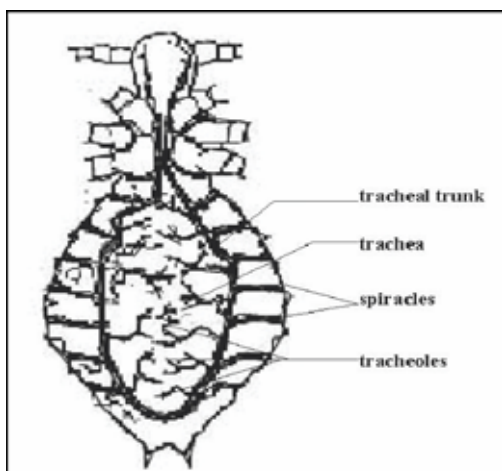


Figure 6.11. Diagram showing an insect spiracle and trachea (From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

goes out via spiracles. This tracheal system also regulates water.

C. Nervous System

The central nervous system consists of a brain connected to a nerve cord, with ganglia occurring at intervals, often one ganglion per body segment (Figure 6.12). Nerves arising from these ganglia reach various parts of

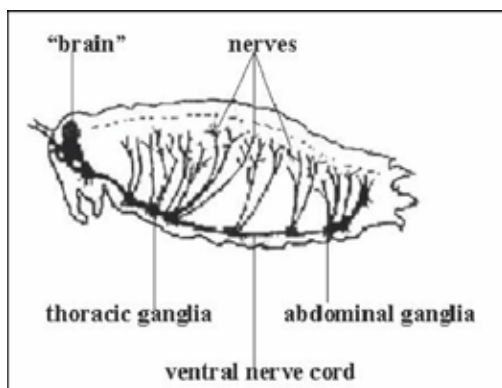


Figure 6.12. Diagram of an insect showing the arrangement of the nerve cord (From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

the body, especially sensory organs like the compound eyes, ocelli, antennae, halteres, palpi, and hairs, serving as sensory receptors.

D. Digestive System

The foregut starts with the mouth and includes the pharynx, esophagus, and proventriculus (Figure 6.13). The posterior part of the esophagus, called the crop, serves as an area for temporary storage of food before it is passed to the midgut for digestion. The muscular proventriculus acts as a valve preventing the food from being regurgitated and may have teeth or spines to aid in the disintegration of food particles. A pair of salivary glands is situated in the thorax. The composition of saliva varies according to the type of insect. In blood-sucking ones, it often contains anticoagulins, which may be allergenic.

The midgut or stomach serves as an area for food storage in the process of digestion and may become greatly distended. It secretes enzymes necessary for insect meal digestion. The beginning of the hindgut is marked by the presence of opaque tubules called Malpighian tubules. The anterior part is called the ileum, while the more distal part is called the rectum, which terminates in the anus.

E. Excretory System

Malpighian tubules act as excretory filters and discharge waste products (Figure 6.13).

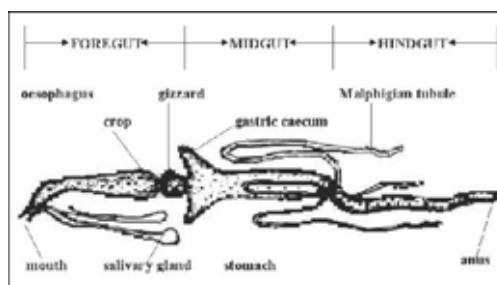


Figure 6.13. The digestive and excretory systems (From Baltazar CR, Salazar NP. Philippine insects: an introduction. Quezon City: University of the Philippines Press; 1979.)

They are milky white to opaque in appearance due to deposition of waste products within their cells.

F. Reproductive System

Insects are dioecious; the male and female must mate before eggs are produced. Insects which lay eggs are called oviparous, while those which deposit larvae are called viviparous.

The reproductive organs of the female (Figure 6.14A) consist of a pair of ovaries which produce eggs and pass them into the oviduct, where they may be fertilized by sperm cells stored in the spermatheca. Some species have accessory glands which secrete an adhesive coating for the eggs.

The male reproductive organs (Figure 6.14B) consist of a pair of testes in which sperm cells are developed. The seminal vesicle serves as storage for spermatozoa until mating occurs. The accessory glands secrete a liquid substance to serve as a vehicle for the sperm cells, which are then passed into the vas deferens and into the penis or ejaculatory organ.

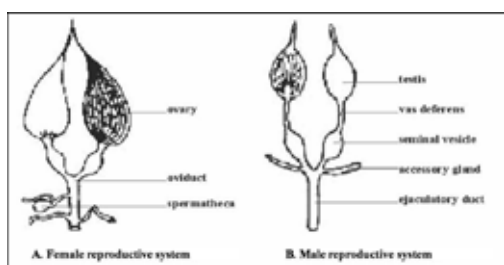


Figure 6.14. Reproductive systems of an insect (From Baltazar CR, Salazar NP. *Philippine insects: an introduction*. Quezon City: University of the Philippines Press; 1979.)

G. The Senses

Insects also possess the senses of touch, taste, smell, hearing, and sight. They also possess other auxiliary senses such as the sense of balance, and possibly orientation.

Because of the chitinized cuticle, the insect's skin is not sensitive to contact. The sense of touch is made possible by sensory hairs connected to a nerve (sensory nerve cell), which is stimulated if hairs are disturbed. Nerve endings are usually concentrated in the mouthparts, antennae, and tarsi.

Taste is usually perceived by the mouth and mouthparts, by the palpi or even by the protarsi. Palps also bear olfactory organs. The sense of smell is highly developed in insects and is used for locating food, finding a mate, and locating a suitable oviposition site.

Insects generally respond only to specific noises, such as the sound made by the wings of a female mosquito. Sound waves may be picked up by fine sensory hairs or by an auditory drum located on the lower part of the insects' front legs. Only some insects, like grasshoppers, cicadas, crickets, and other species of moths have "ears" or tympanic membrane. Flies and mosquitoes are believed to hear by means of a cup-like organ on the second antennal segment, which responds to sound waves picked up by the rest of the antennae.

The principal organs of sight are the compound eyes and ocelli. Insects cannot move nor focus their eyes. It is not possible for insects to see a sharp clear image, and they are only able to see blurred images. These eyes are provided with nerves, which transmit stimuli to the brain.

Because of these different senses, insects are able to react to their environment. Their responses arise from simple stimuli, such as light, heat, gravity, hunger, and smell. Their reactions consist of more or less fixed behavioral patterns and they react similarly to the same stimulus. This is called automatic behavior, which does not involve reasoning. With insects, behavioral reactions are usually immediate. Although the brain is located in its head, each of the body regions act independently, or in a semi-autonomous manner, because pairs of nerve centers called ganglia are located along

the bottom side of the insect's body and are connected to the brain by a nerve cord.

Arthropods can cause direct and indirect injuries to humans. Below is the list of medically important classes and orders under Phylum Arthropoda. Discussion of their important features and roles in human disease are given in the next two sections.

A. *Class Insecta*

Order Diptera (mosquitoes and flies)

Order Siphonaptera (fleas)

Order Hymenoptera (bees, wasps, and ants)

Order Lepidoptera (moths and butterflies)

Order Hemiptera (bed bugs and kissing bugs)

Order Anoplura (sucking lice)

Order Coleoptera (beetles)

B. *Class Crustacea*

These arthropods are aquatic in nature. Their bodies are divided into two: cephalothorax (head and thorax fused together) and abdomen. Respiration is either by means of true gills or directly through the body wall.

There are two orders of medical importance:

Order Copepoda (cyclops)

Order Decapoda (macrocrustaceans, e.g., crabs, lobsters, and shrimps)

C. *Class Arachnida*

These arthropods are both aquatic and terrestrial in nature. Their bodies are divided into a cephalothorax and abdomen. The cephalothorax bears six pairs of appendages: anterior chelicerae, pedipalps, and four pairs of walking legs.

There are three orders of arachnids which are of medical importance:

Order Scorpionida (scorpions)

Order Araneida (spiders)

Order Acarina (mites and ticks)

D. *Class Chilopoda (centipedes or hundred-legged worms)*

These arthropods are terrestrial, elongated, and have many segments. The body is dorsoventrally flattened with a pair of legs on each body segment. The appendages of the first body segment are modified to serve as poison claws.

E. *Class Diplopoda (millipedes or thousand-legged worms)*

These are terrestrial, elongated and have many segments. The body is cylindrical with two pairs of legs per body segment. There are no poison claws. They do not bite humans, but secrete substances that are irritants to human skin.

F. *Class Pentastomida (tongue worms)*

Adults have elongated bodies which are either flattened (e.g., *Linguatula* in dogs) or cylindrical (e.g., *Armillifer* in pythons). In *Armillifer*, the body is divided into a series of unusually conspicuous rings, which are not true segments. This characteristic raises questions on whether this class should be under Phylum Arthropoda. The larval stage, however, is segmented. The adults usually live in the lungs or air passages of their hosts, while larvae live free or encysted in the viscera of some other hosts.

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Arthropods as Direct Causes of Injury

Lilian A. de las Llagas

Ways by which Arthropods Affect Humans

The direct effects of arthropods on humans are generally classified as: (a) envenomization; (b) ectoparasitism; (c) ingestant and inhalant allergens; (d) food, water, and house pests; (e) myiasis; and (f) entomophobia and delusory parasitoses (Table 6.2).

Table 6.2. Specific injuries and their causative agents

Injury	Agents
Envenomization	Venomous arthropods: bees, wasps, kissing bugs, ants, caterpillar, centipede spider and scorpion
Ectoparasitism	Non-venomous arthropods: mosquito, flea, lice, mite and ticks
Inhalant allergens	Dead/decomposing bodies of insects: cockroach feces, hairs and spines, house dust mites (HDM)
Ingestant allergens	Mites, cockroach feces, larval stages of small beetles
Contact allergens	Urticating caterpillar hair, blister beetle, millipede
Food and water pests	Moth, beetle, mites, chironomids, maggots
House pests	Mosquitoes, flies, cockroaches
Myiasis	Fly maggots feeding on human wounds

Envenomization

Venoms are poisonous substances, which certain animals secrete and introduce by biting or stinging. Arthropod venoms are usually poisonous when they are injected through the integument, or come in contact with injured skin. The toxic effect of the injected venom depends upon its chemical composition and the amount injected. Allergic reactions may

follow repeated exposure to various venomous arthropods. Arthropods that cause direct injury through envenomization are described below.

A. Order Hymenoptera (bees, wasps, and ants)

The name of the order comes from the Greek word *hymen* meaning membrane and *ptery* meaning wing. These are, therefore, membranous-winged arthropods. Their mouthparts have strong jaws, which are adapted for biting. Typically, there are two pairs of wings, with the hind pair being smaller than the front pair. The wings are folded back over the abdomen when at rest. The body is divided into three segments: head, thorax, and abdomen. The abdomen is further divided into abdominal segments, but usually only six or eight are evident. The last abdominal segment is a modified ovipositor, the stinging apparatus of a female hymenopteran. This modification of the egg-laying tube enables it to function as a very efficient weapon for both offense and defense. The sting is withdrawn into the body when not in use. The presence of an ovipositor serves to identify the female since the sting is absent in the male.

The stinging hymenopterans are divided into two distinct groups: those that kill their prey by stinging, and those that sting only to paralyze their prey.

Formic acid, which causes the paralysis, can be found at the base of the stinger of some hymenopterans. The apparatus of the hymenopteran that kills has an acid gland opening directly into the poison sac, and an alkaline gland, which is comparatively small. It is the combination of these acid and alkaline fluids that results in the death of the prey or causes extreme pain.

Stinging hymenopterans, which have been found responsible for adverse reactions in humans, are members of superfamilies Apoidea (bees) (Plate 6.1), Vespoidea (wasps, hornets, and yellow jackets) (Plate 6.2), and Formicoidea (ants).



Plate 6.1. Bee (*Bombus* sp.)
(Courtesy of Dr. Lillian de las Llagas)



Plate 6.2. Wasp
(Courtesy of Dr. Lillian de las Llagas)

1. *The Stinging Apparatus (modified ovipositor)*

The venom apparatus consists of three parts: the piercing apparatus, the lateral plate and appendages, and the poison sac and glands. If the ovipositor stinger stays in the wound, one can be sure it is from a honeybee. The stinger of the honeybee is barbed; when it is pulled from the insect, the honeybee dies. The honeybee, therefore, is not capable of multiple stings, unlike the hornets, wasps, and bumblebees, which all have unbarbed stingers (Plate 6.3).

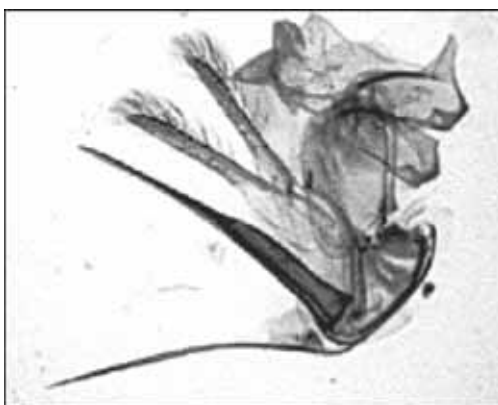


Plate 6.3. Bee stinger
(Courtesy of Dr. Lillian de las Llagas)

Among ants, the bite may be supplemented by the sting. Formic acid of the formicine ants may reach intra- or sub-dermal tissues only through wounds made by the mandibles. Some ants bite and sting simultaneously. The bite is a necessary mechanical advantage for inserting the sting. Salivary secretions are not introduced.

2. *The Nature and Action of the Venom*

Venom secretion in worker honeybees begins just prior to emergence and increases slowly toward a maximum amount between the 10th and 16th day. This amounts to 0.3 mg of liquid or 110 pg of venom. Secretions cease after 20 days. Protein food, mostly pollen, is required for the full production of venom. Electrophoretic and chromatographic studies

have shown that bee venom contains histamine, which is released in the tissues. Histamine, however, is not the major pharmacological component. Bee venom appears to contain no cholinesterase or 5-hydroxytryptamine, but it does contain low molecular weight ninhydrin-reacting compounds, the action of which is not completely understood.

Toxic effects of bee venom result from the combined actions of mellitin, phospholipase A, and hyaluronidase. These account for both the local and general reactions such as pain, inflammation, swelling, and redness. Phospholipase is also known to indirectly cause hemolysis of red blood cells.

The initial response to the venom is a wheal and flare at the site of the sting, then itching and flushing follows. As the venom circulates in the blood, more widespread symptoms occur. Vascular effects (hypotension), and then pulmonary effects, with asthma or angioedema in the airways, are observed.

The effects of stings are two-fold: the direct toxic effect, and the anaphylactic shock, which may develop in those who become sensitized to it. Many beekeepers become desensitized to bee venom as a result of repeated stings. The sera of beekeepers contain antibodies to phospholipase A.

B. Order Hemiptera

Family Reduviidae consists of insects described as “cone-nosed” because they have narrow pointed, cone-shaped heads. They are known as “assassin” bugs or “cannibal” bugs which prey upon soft-bodied insects, and “kissing” bugs because some biting species attack the face.

Subfamily Triatominae feeds on the blood of vertebrates, including humans. Members of this subfamily include the genera *Rhodnius*, *Triatoma*, and *Panstrongylus* (Plate 6.4).

Subfamily Hespatoriinae also inflict painful bites, but they are not necessarily



Plate 6.4. Kissing bug (*Triatoma* sp.)
(Courtesy of Dr. Lillian de las Llagas)

bloodsuckers. Members include the genera *Arilus* and *Reduvius*.

Triatomines are differentiated by the position of antennal insertion. In *Rhodnius*, the insertion is at the top of the head. In *Triatoma*, the insertion is midway between the compound eyes and the tip of the head, while in *Panstrongylus*, the insertion is near the compound eyes.

Genus *Triatoma* has been reported to inflict painful bites (Plate 6.4). *Triatoma rubrofasciata* bite was first reported in the Philippines by Africa in 1934. There have been periodic complaints from patients bitten by this bug. Major complaints include swelling in the area of the bite, nausea, vomiting, irritation, and pain. The extent of reaction to these bites appears to depend on the sensitivity of the host and on the amount of antigen injected. Symptomatic reactions are probably due to the phenomenon of sensitization rather than a response to a primary irritant. Bugs usually bite at night and the lesions are usually in the exposed parts of the body. The venom is probably the same as the venom of bees and wasps.

Arius is called the wheel bug because of a cog-like crest found on its thorax, which is its distinct feature. The proboscis has three long and stout joints. When not in use, the proboscis is bent ventrally under the head. This bug is usually found in vegetation, near rocks, other outside debris, and inside buildings when raining. This bug is known to feed on other arthropods.

The wheel bug attacks humans as a form of defense, particularly when its resting place is disturbed. Its bite inflicts severe and immediate pain.

C. Order Lepidoptera

The larvae of moths and butterflies are called caterpillars (Plates 6.5–6.6). They usually have a cylindrical, worm-like body which is divided into 12 segments: the first three make up the thorax, and the other nine the abdomen. The well-developed head bears a mouth, 12 tiny eyes and two very short antennae. The mouthparts of the larvae consist of strong biting jaws and mandibles adapted for biting. This differs from the adults, which have sucking mouthparts. Some larval species have spines or hairs, which may contain toxin. In some instances, irritation seems to be largely due to a

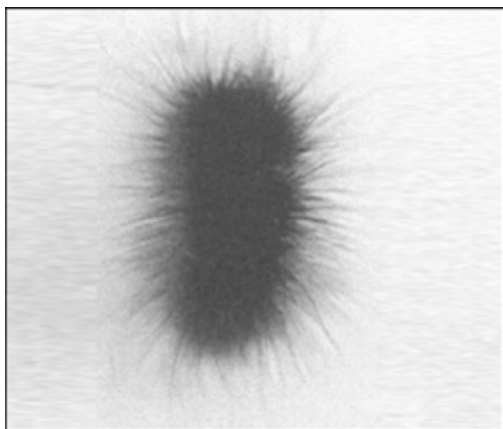


Plate 6.5. Caterpillar, dorsal view
(Courtesy of Dr. Lillian de las Llagas)

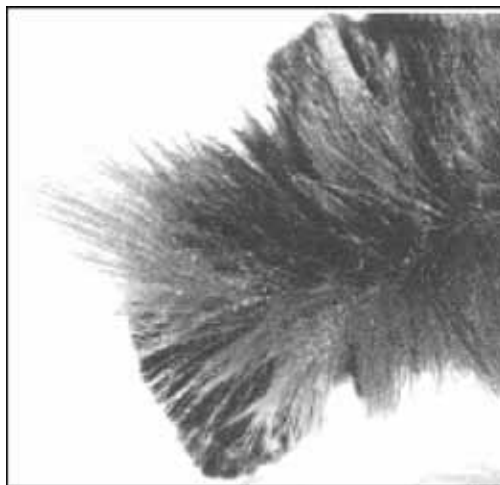


Plate 6.6. Caterpillar head and thorax, lateral view
(Courtesy of Dr. Lillian de las Llagas)

mechanical effect, similar to that of glass fibers. The hairs are of several kinds and many of them are barbed, so that they tend to stick to the skin. Upon contact, the susceptible individual may experience a burning sensation on the affected skin, which may show redness or inflammation. Other areas may show urticarial wheals.

If the hairs get into clothing, widespread dermatitis may occur. Wind-blown hairs in drinking water can also cause inflammation of the mucous membrane of the mouth.

D. Class Chilopoda

Centipedes are terrestrial arthropods that are dorsoventrally flattened, and have one pair of legs per body segment (Plate 6.7). The head bears a pair of long antennae, a pair of mandibles, and two pairs of maxillae (Plate 6.8).

The first body segment bears a pair of modified legs found just ventral and lateral to the mouth. These modified legs form claws, the terminal joints of which are curved, sharply pointed, horn-like fangs that connect to the venom glands.

Large species of centipedes can grow up to 25 cm in length and are considered venomous.



Plate 6.7. Centipede
(Courtesy of Dr. Lilian de las Llagas)

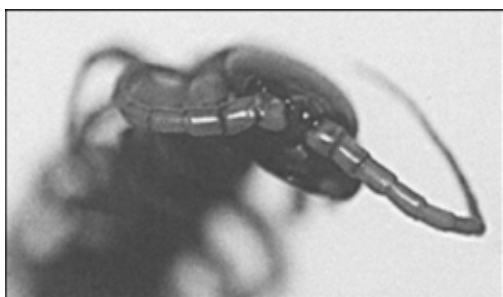


Plate 6.8. Centipede head
(Courtesy of Dr. Lilian de las Llagas)

Small types, about 2 to 5 cm long, are harmless, since they do not have well-developed fangs for biting. The amount of venom introduced depends on the size of the centipede.

The bite is characterized by local pain at the site of puncture, hardening of the skin, formation of papules, rash, swelling, and purple patches. However, each sign or symptom subsides within 24 hours if the wound remains uninfected.

Pineda, in 1934, reported a death due to a centipede bite in the Philippines. The immediate reactions noted after the bite were pain, numbness of the affected area, and a minute, reddish puncture wound. Noteworthy were the following: (a) proximity of bite to the

brain where marked congestion was observed indicating the concentration effect of the poison, and (b) thinness of the skin in the region of the bite, which allowed the deep injection of a large amount of poison and its rapid absorption.

E. Order Scorpionida (scorpions)

The body is divided into a cephalothorax and an abdomen. The cephalothorax is unsegmented and covered by a dorsal plate, called a carapace which contains 2 to 12 eyes. The abdomen is segmented with the terminal five segments ending in a bulbous sac and a conspicuous stinger. The sac contains two poison glands which are connected to the terminal stinger by ducts. Scorpions have no antenna but their bodies and legs are covered with sensory hairs (Plate 6.9).

Scorpions are nocturnal creatures. During the day, they remain hidden under stones, logs, piles of lumber, closets, shoes, folded blankets, folded papers, and other debris. They come out



Plate 6.9. Scorpion
(Courtesy of Dr. Lilian de las Llagas)

of their hiding places at night to obtain food, consisting mainly of insects and other arachnids.

Although scorpions rarely sting humans, they are considered dangerous since they produce hemolytic and neurotoxic venom. Investigators have described the venom to be protein in nature, and its toxicity is dependent on sulfhydryl groups. Hemolytic venom causes painful swelling at the site of the sting, which diminishes within 30 minutes. Neurotoxic venom may produce numbness at the sting site, profuse sweating, salivation, nausea, and paresthesia of the tongue. Drowsiness may follow the immediate sharp pain. It has been observed that if the victim is alive for three hours after the sting, survival is probable. No other arthropod produces these symptoms.

A. Order Araneida (spiders)

The body is divided into a cephalothorax and an abdomen joined by a slender “waist,” called a pedicel or stalk. The cephalothorax commonly has eight simple eyes and six pairs of appendages. The first pair of appendages, the chelicerae or fangs, are claw-like and utilized by the spider to capture its prey. The second pair of appendages is a pair of six segmented palpi or pedipalps, which are found in front of the legs and are sometimes mistaken for legs. The other four pairs of appendages are walking legs.

The chelicerae are segmented appendages and have hollowed tips, through which the venom is injected from the modified salivary or poison glands.

The spinning organs are located near the back of the abdomen and on the underside. There are usually six spinnerets used for spinning the web.

Spiders are cosmopolitan in distribution and nocturnal in habit. They prefer quiet, cool shelters and dimly lit areas.

Most spiders are harmless. Few have chelicerae that are strong enough to penetrate human skin. Among the dangerous species are *Latrodectus* (black widow spider or “katipo”)

and *Loxosceles* (brown widow/recluse spider). The females of both spiders destroy or kill the males after mating. Thus, they are called “widow spiders.”

1. *Latrodectus*

The mature female black widow spider is deep black in color. It has red markings in the form of an hourglass on the underside of its abdomen. It is approximately 1.2 to 5.1 cm in size (Plate 6.10).



Plate 6.10. Black widow spider (*Latrodectus hasselti*) (Courtesy of Dr. Lilian de las Llagas)

A bite from the black widow spider is often inconspicuous. Slight local swelling and two tiny red spots may appear, with local redness usually evident at the point of attack. Within a few minutes after the bite, latrodectism develops, characterized by severe pain which spreads throughout the extremities and the trunk. Within a few hours, chills, vomiting, cramps, delirium, and spasms may occur. Abdominal pains are frequently severe. These symptoms may be mistaken for appendicitis, colic, or food poisoning. In 1987, Grace and DaÔgo reported a case of spider bite by a spider popularly known as “gagambang gubat.” The patient exhibited contraction of leg muscles, high fever, hemoglobinuria, and jaundice.

The venom apparatus consists of two glands, located on the cephalothorax, which are

connected by ducts to two curved fangs, located on the distal segments of the chelicerae. The venom is a complex protein with a neurotoxic lipoprotein fraction.

2. *Loxosceles*

This species may be distinguished from other forms by its three pairs of eyes arranged in a semi-circle fashion on the forepart of the head, and a dark violin-shaped marking immediately behind the simple eyes. This is more commonly found inside houses than the black widow spider.

Loxoscelism is caused by this spider's bite. Although symptoms are localized, it differs from the bite of the black widow in that the initial thick wheal may become necrotic. In 1987, Barrion reported two cases of loxoscelism. Two boys were bitten on their hands by a spider while climbing a mango tree. The immediate reactions were localized swellings at the bite sites, high fever, and contractions of leg muscles. Later, necrosis and gangrene were observed on the bitten areas. The venom of *Loxosceles* may contain a spreading factor and this may be responsible for the necrotic effect.

Ectoparasitism and lesions due to arthropod bites

A. Order Diptera (Class Insecta: mosquitoes and flies)

This order is characterized by the presence of a single pair of wings. The second pair is reduced to small knob-like structures called halteres, which are used during flight as balancers. There are three suborders of medical importance:

1. Suborder Nematocera (e.g., mosquitoes, blackflies, midges, and sandflies).

Insects under this suborder possess a pair of thread-like antennae of similar segments. There are about 11 to 15 segments for the long type of antennae. These antennae are longer than the head and thorax combined. The mouthparts are adapted for sucking blood.

a. Family Culicidae (Mosquitoes)

Among the mosquitoes, only females bite, but both sexes feed on nectar and juices. The sexes can be easily differentiated by looking at the antennae. The male has a hairy or plumose antenna, while the female has pinnose antennae with less hair.

Mosquitoes have scaly wings, the third vein of which is simple, while the second and fourth veins are branched. The mouthparts belong to the piercing-sucking type. Mosquitoes are about 4 to 6 mm in length. Some small-sized species measure about 2 to 3 mm in length, while the larger-sized species can be as long as 10 mm. Mosquitoes have two compound eyes that are made up of many facets. Just below the antennae is a pair of palps, dilated or pointed at the tips, depending on the species.

The thorax is slightly humped and is covered dorsally and laterally with scales. The abdomen is composed of 10 segments but only the first 8 are visible. The last abdominal segment of female mosquitoes terminates in a small pair of cerci, whereas, in the males, a prominent pair of claspers is present.

Two major divisions of Family Culicidae are Anophelinae and Culicinae. Anophelines include the *Anopheles* mosquito, whereas *Aedes*, *Culex*, *Mansonia*, and *Armigeres* mosquitoes are culicines.

i. Mechanism of Bite Reaction

Some species bite during the day, while others hide and become active at night, dusk or dawn. Bites are usually inflicted on exposed body surfaces. The reaction to these bites may either be immediate or delayed or sometimes both, depending on the frequency of contact. There are three general types of reactions:

- Hemorrhagic macule. There is a punctum seen at the site of the bite, which may develop without symptoms of irritation; in the course of several days, these marks become darker and eventually disappear.

- Delayed reaction papule. This may be observed from a few hours up to 2 weeks after the bite; there is swelling accompanied by intense irritation.
- Immediate reaction wheals. These appear within a few minutes of the bite, but do not last long, usually less than an hour; these cause moderate irritation.

The reactions to mosquito bites are associated with the trauma produced by the mechanical insertion of the proboscis by the mosquito. The initial cutaneous reaction is due to the sensitizing effect of the saliva. The saliva chemically consists of histamines and 5-hydroxytryptamine, or kinin.

b. Family Simuliidae (*Simulium* or "black flies")

These are humpback dipterans measuring 1.5 to 4.0 mm long. They are usually black in color, but may sometimes be gray. They have short legs and short antennae. The mouthparts are short and relatively inconspicuous (Plate 6.11). Only the females bite, though their mouthparts do not penetrate the host's deeper tissues. These dipterans usually stay near vegetation. Its intermediate stages breed in fast flowing streams.

The lesions produced are characterized by localized swelling and inflammation, accompanied by an intense irritation, which lasts for several weeks.

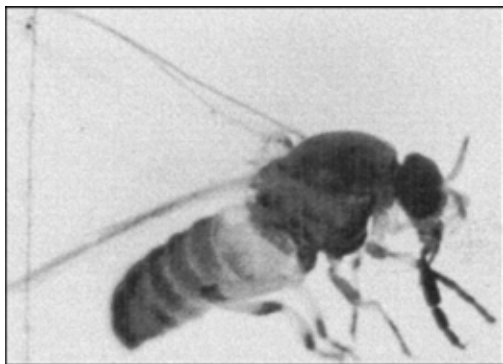


Plate 6.11. Blackfly (*Simulium* sp.)
(Courtesy of Dr. Lillian de las Llagas)

c. Family Ceratopogonidae (Leptoconops, Culicoides, midges, "nik-nik")

These insects are small, about 1.5 to 5 mm long. The antennae are long, consisting of about 15 segments. The wings are spotted and covered with hairs. The mouthparts are short, relatively inconspicuous, and are not projected forward (Plate 6.12). Males do not take blood meals. Females stay around vegetation, cow sheds, muddy debris, and shaded trees. The eggs are laid on the surface of mud, wet soil, cow dung, and other habitats that are moist or partially submerged in water. Midges usually swarm over the head, biting the face and neck, and exposed body parts. Lesions are usually in the form of multiple vesicles, which produce intense itching.

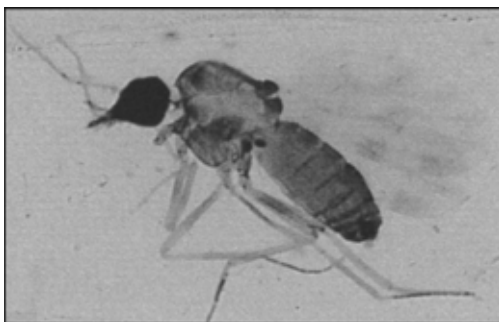


Plate 6.12. Midge (*Culicoides* spp.)
(Courtesy of Dr. Lillian de las Llagas)

d. Family Psychodidae (Phlebotomus, sandfly, mothfly)

These flies are small, about 2 to 5 mm long. The body and wings are entirely covered with hairs, thereby giving them the appearance of small moths. The wings are lanceolate in shape and have simple wing venation. The antennae have 12 to 16 segments. The legs are long and slender. The mouthparts are short and inconspicuous (Plate 6.13). Only the females bite, feeding at night. They hide in dark corners during the day. They usually attack the face and the neck, and produce vesicles or wheals. Intense itching, pain, heat, and swelling occur. A blue scar often remains.



Plate 6.13. Sandfly (*Phlebotomus* spp.)
(Courtesy of Dr. Lillian de las Llagas)



Plate 6.14. Horsefly (*Tabanus* spp.)
(Courtesy of Dr. Lillian de las Llagas)

Eggs require a moist environment with high humidity, such as holes in the ground and leaf litters.

2. Suborder Brachycera (e.g., horseflies and deerflies)

The antennae are shorter than the head and thorax combined, and is composed of three segments. The third segment is enlarged and bears a terminal bristle called the style. The mouthparts belong to the cutting-sponging type.

a. Family Tabanidae (*Tabanus* and *Chrysops*)

These flies vary in size depending on the species. They can be smaller than a housefly, or they can be very large, measuring 5 to 25 mm. *Tabanus* (horse fly) is uniformly black but has whitish markings on the thorax and abdomen. Its wings are clear (Plate 6.14). *Chrysops* (deer fly) is smaller than the horse fly and has a more rounded head. The middle part of its wing is patterned with a brown coloration. Males of these flies do not bite. Eggs are deposited on the underside of leaves, twigs, stems, stones, and rocks overhanging or adjacent to their larval

habitat, where the environment is moist. Most species are aquatic or semi-aquatic.

Because of their mouthparts, these flies inflict very painful bites, resulting in erythema and swelling. Their attacks are usually persistent, producing multiple painful non-pruritic lesions on exposed areas.

3. Suborder Cyclorhapha/Orthorhapha (e.g., houseflies, *Stomoxys*, "biting housefly," and other biting flies)

The antennae consist of three segments. The third segment is enlarged and carries a conspicuous bristle called the arista. The mouthparts are of the sponging and piercing types.

This fly resembles the housefly (*Musca*) very closely, but differs from the housefly by having a piercing-sucking type of mouthparts. It has four brown-black longitudinal bands on its thorax, and its antennae are of the aristate type. It breeds in moist, rotting, and fermenting vegetable matter, such as grass, hay, or horse manure. Both males and females suck blood. They are active at daytime and bite outdoors. They inflict very painful bites.

B. Order Anoplura (sucking lice)

These are wingless permanent ectoparasites of mammals. They measure 1.5 to 3 mm in length. The body is dorsoventrally flattened and usually gray in color. Lice are strictly host-specific. Head lice and genital lice, for example, are seen only in humans. They do not infest domestic household pets and other animals. Both species belong to the family Pediculidae, having mouthparts adapted for piercing-sucking.

Pediculus humanus capitis is also called the head louse. The male measures 2 to 3 mm and the female 3 to 4 mm in length. Its head is small compared to its body size. It is narrow and pointed in front, and has antennae with four to five segments. Its legs are of the clinging type and are of equal size. It is found on the scalp (Plate 6.15).

Head lice infestation is very common in the Philippines. Children are most commonly affected. This condition is very much associated with warm weather, as the lice require this for

further development. Severe infestations may result in the hair becoming matted with eggs. Itching is usually the predominant symptom.

The itching is attributed to the injection of the saliva, and may also be a reaction to lice feces. The intensity of itching varies from one person to another, and this is highly correlated with the degree of infestation.

Phthirus pubis (genital louse, pubic/crab louse) has a crab-like body. It is nearly as broad as it is long and measures about 1.5 to 2 mm. The middle and hind legs are stouter than the first pair. Pubic lice infestation is more common in adults rather than in children (Plate 6.16). Transmission usually results from intimate contact. Ordinarily, the pubic louse confines its activities to pubic hairs, but it may also be found in other parts of the body where hair is coarser, such as axillary hair, eyebrows, or eyelashes.

Patients with pubic lice infestation were found to be concomitantly infected with sexually-transmitted infections (e.g., gonorrhea). Infestation with this louse is commonly associated with complaints of intense pruritus in the affected region due to the presence of nits or eggs.

Other lice include *Haematopinus* (hog louse), *Trichodectes canis* (dog biting louse),



Plate 6.15. Louse (*Pediculus humanus capitis*)
(Courtesy of Dr. Lilian de las Llagas)

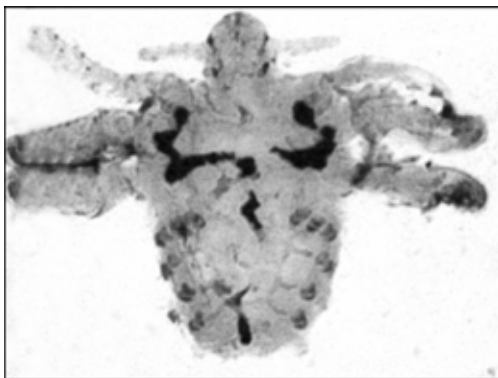


Plate 6.16. Pubic louse (*Phthirus pubis*)
(Courtesy of Dr. Lilian de las Llagas)

Linognathus (cattle louse), *Menopon* (chicken louse), and *Columbicola* (pigeon louse). These are lice of domestic animals, and they do not attack or infest humans.

All lice have similar life histories. The adult lays eggs, which are called nits. These appear as white or gray oval bodies which are glued to the hair by the head, or by the gonopod, as seen in pubic lice. The young resemble the adults, except in size. They require at least 1 week to complete development.

C. Order Siphonaptera (fleas)

These are wingless insects measuring less than 4 mm, usually 1.4 to 2 mm in length. The body is laterally compressed and covered with spines which enable them to move freely. The antennae are short, three-segmented, club-shaped, and embedded in a deep groove. The legs are adapted for jumping, allowing them to jump as far as 28 cm vertically or 32 cm horizontally. On smooth surfaces, they progress by means of short jumps and running. Both sexes feed on blood. The mouthparts are adapted for piercing and sucking. Compound eyes are lacking. Some species, however, possess degenerate eyes without distinct facets, while others are completely blind. In some species, a conspicuous row of spines or a “comb” is present. This is useful in recognizing the different species of fleas.

The most common species are *Ctenocephalides canis* (dog flea), *C. felis* (cat flea), *Pulex irritans* (human flea), and *Xenopsylla cheopis* (rat flea). Although *Ctenocephalides* preferably feed on dogs and cats, they can also bite humans when their preferred hosts are not around.

Fleas remain on their hosts less constantly than lice do. Female fleas, after blood feeding, lay their eggs on the fur of the hosts, in dust, on debris, in floor cracks, and under rugs and carpets. The larvae feed on organic debris. They usually avoid light. Pupae emerge after 10 to 12 days and may remain inactive for some

time. Any change in humidity, temperature, or vibration stimulates the pupae to escape from their cocoons and enable them to emerge as adults. As fleas suck blood from their hosts, they inject saliva to prevent the host's blood from clotting. This secretion contains amino acids, peptides, ketones, low molecular weight sugars, polyhydric alcohols, phenols, aldehydes, and phosphates, all of which are capable of inducing sensitivity in the host.

Bites appear as small punctures, which represent areas probed by the fleas. Initially, the flea explores the exposed skin area completely, frequently stopping to probe the surface without necessarily feeding at each probe site. Once a suitable site is selected, the flea bites and remains attached. It then moves along, biting and feeding in a grouped but irregular pattern, resulting in multiple lesions. Grouping, therefore, is one of the most distinct descriptions of the lesions. Appearing immediately around the probe site is a wheal with or without accompanying erythema. Aside from the presence of multiple zigzag lesions, the diagnosis of flea bites is also confirmed by previous exposure to animal hosts.

D. Order Hemiptera (bed bugs)

Cimex hemipterus is common in tropical climates. Bed bugs inflict very irritating and itchy bites. On examination, multiple bite lesions are found with erythematous wheals of uniform size with red punctate centers that persist for many days. The skin condition caused by a *Cimex* bite is called cimicosis. Bed bugs are generally nocturnal feeders (Plate 6.17).

The bug uses its beak-like proboscis, with its mandibles and maxillae, to pierce or puncture the skin of the host. It feeds directly from the capillaries. The combination of initial skin piercing, and the subsequent probing for blood, results in swelling and irritation. It is reported that the amount of saliva injected by the bed bug is around 0.16 µL. This saliva contains an anticoagulant.



Plate 6.17. Bedbug (*Cimex* sp.)
(Courtesy of Dr. Lillian de las Llagas)

E. Order Acarina (mites and ticks)

The majority of mites and ticks (Table 6.3) are round or oval, dorsoventrally depressed forms with the head, thorax, and abdomen fused together, lacking visible segmentation. The anterior portion is modified to form a capitulum, made up of a central hypostome, and paired chelicerae and palpi, used for attachment and obtaining food.

Table 6.3. Principal differences between mites and ticks

Parameter	Mites	Ticks
Body	With long hair	With short hair, or may be bare
Hypostome	Hidden and unarmed	Exposed with teeth
Size	Usually small (microscopic)	Large (macroscopic)
Body texture	Membranous in appearance	Leathery in appearance
Pedipalps	Almost lacking in segmentation	Prominent and distinctly segmented
Chelicerae	Reduced to blades and rods	Heavily chitinized, bearing teeth at their distal ends

1. Mites

a. Chigger infestation

Chigger infestation is caused by the larval stage of *Leptotrombidium* species. The larvae feed on the host's epidermal cells. Infestation usually occurs when one walks through long grass, or when one sits or lies on infested ground. Chigger bites cause intense pruritus and severe reactions may also occur.

The larval chigger is very small, about 0.15 to 0.3 mm long depending on the species. The larva may increase its size six-fold after feeding. It is usually reddish-orange, but may be pale or yellow. There are three pairs of legs covered with fine hairs. It does not burrow into the host skin; but merely attaches itself using its large, segmented palps and blade-like chelicerae. It secretes powerful digestive enzymes, which liquefy epidermal cells, and the resultant fluid serves as its main diet.

Although the chigger larva drops off the host soon after feeding, the host response may persist for weeks. Itching begins a few hours after the chigger has attached itself to the skin, which transforms the affected area into a wheal. In heavy infestations, a patient may find it almost impossible to sleep because of the intense pruritus, since the heat of a warm bed may intensify the itching. Pruritus gradually decreases and resolves after 5 to 14 days. The chigger usually attacks the legs, or attaches itself to skin in areas where skin meets clothing, such as the edges of a brassiere, the waistband of underwear, and the tops of socks.

Diagnosis of chigger infestation can usually be made on the basis of a history of previous outdoor exposure, and typical skin lesions in areas where clothing is snug.

b. Scabies

Scabies ("galis-aso") is caused by *Sarcoptes scabiei*. It is a contagious skin infection. Infestation with this mite is seen in all age

groups, and is very common in crowded dwellings. The usual transfer of the mite is by direct contact. The variety of *S. scabiei* that causes sarcoptic mange in dogs can also burrow in human skin but stays only for a limited duration. The mite causes intense pruritus that is more severe at night and may persist for some time.

The female mite is 0.3 to 0.45 mm in length. It is whitish, disc-shaped, and flattened ventrally. The mite is covered with membranous, small, peg-like protuberances, has a few bristles, both dorsally and ventrally. The mite has a few lines across the body, giving it a striated appearance.

The female mite favors places on the body where the skin is wrinkled, such as wrists, elbows, feet, penis, scrotum, breasts, axillae, and in between fingers. Using its short, stout, sharp pincer-like chelicerae, the mite digs and eats its way through the surface of the stratum corneum. It buries itself, excavates, and creates a tunnel then feeds on liquids oozing from dermal cells. During the mite's progress along the tunnel, it lays about four to six eggs and sometimes defecates while feeding.

Definitive diagnosis is by demonstration of the female mite. Physical examination of the patient reveals mite burrows. In chronic cases, the skin becomes eczematoid.

c. *Demodex folliculorum* and *Demodex brevis*

Demodex folliculorum (on face) and *Demodex brevis* (on face and trunk) cause follicle mite infestation. These mites are found in the hair follicles and sebaceous secretions of humans. They are sometimes present on the skin and usually cause no severe symptoms. These mites, especially *D. folliculorum*, are associated with "black heads." On rare occasions, the mites produce an erythematous follicular eruption in the beard area of men.

The adult mite is usually less than 0.5 mm in length, and is worm-like and elongated in appearance. The thorax bears eight very short,

stumpy legs, and the abdomen is annulated. Other than *Demodex*, infestation may also be caused by *Dermanyssus* (red poultry mite), *Ornithonyssus* (tropical rat mite), *Pyemotes* (grain itch mite), and *Acarus* (cheese mite).

- *Dermanyssus*. This mite is known to attack humans, but is actually a common parasite of wild birds. It feeds on blood, causing irritation and discomfort. Its common name is derived from its ability to thrive in poultry houses. The adult is about 1 mm long and its red color is due to ingested blood. The mouthparts are modified for piercing and sucking.
- *Ornithonyssus*. This mite attacks people living in rat-infested buildings, like dormitories, restaurants, warehouses, and granaries. It is capable of inflicting a bite that is irritating and painful. *Ornithonyssus* generally resembles *D. gallinae*, and is also red after a blood meal.
- *Pyemotes*. People handling infested grain, cotton, and hay may develop dermatitis due to this mite. The adult is about 0.2 to 0.3 mm, and is whitish or yellowish. The female has a pair of club-shaped setae between its first and second pair of legs. The male is about 0.16 mm, has a broader body, and has no club-shaped setae on its thorax.
- *Glycyphagus*, *Acarus*, *Tyrophagus*. These stored product mites cause dermatitis in humans often called miller's, grocer's, copra, or worker's rash, depending on the material being handled. These parasites can also precipitate an attack of bronchial asthma. These mites are about 0.4 to 0.5 mm long. They are whitish or pale yellow in color and resemble *Pyemotes* mites, though their chelicerae are large, and the setae on their bodies are longer and more conspicuous.

2. Ticks

Two types of ticks bite humans: soft or Argasid, and hard or Ixodid ticks. Hard ticks, which are difficult to remove, are more frequently encountered. Ticks are readily distinguished from insects by their strongly fused thorax and abdomen. The body is ovoidal and is capable of great expansion, particularly in females. There are six legs in the larval stage, and eight in the nymphal and adult stages. Ticks are bigger than mites and are usually more than 1 mm in length. The head bears the mouthparts, which consist of two small, retractile mandibles or chelicerae, a pair of short palpi, and a well-developed hypostome armed with teeth.

Generally, ticks pass through the egg, larval, nymphal, and adult stages over months or years. Eggs are usually laid on the ground in batches of 100 to 18,000. The larvae emerge and climb up any available object in order to reach passing hosts. Ticks of some species remain on the same host until they reach maturity, but others find two or more hosts for their blood meal. Females take prolonged blood meals lasting for 8 to 10 days. Males, however, remain attached to the host only for a few hours in order to mate with females.

Once the tick comes in contact with a host, the hypostome and chelicerae are inserted into the skin. Using their recurved teeth, a firm hold is maintained, reinforced by a cement-like secretion. The tick can detach quickly once it is fully engorged without the host noticing it. Forceful removal of the tick may result in granuloma formation which may persist for days or even months after the bite. This granuloma may be due to either a reaction to mouthparts, or to injected salivary secretions. The granuloma measures 0.5 to 2 cm.

Tick paralysis is an acute disorder of the central nervous system, and is believed to be caused by a neurotoxin secreted by the salivary gland of many species of ticks in the process of prolonged feeding.

Inhalant Allergens

Decomposed and pulverized arthropods, cast skin, scales, hairs, spines, cocoons, and webs permeate the air via upward air streams and convection currents, and are thus considered inhalant allergens of humans (Plate 6.18). Their relationship to inhalant-respiratory allergic disease has been the subject of interest of many workers in the field of allergology.

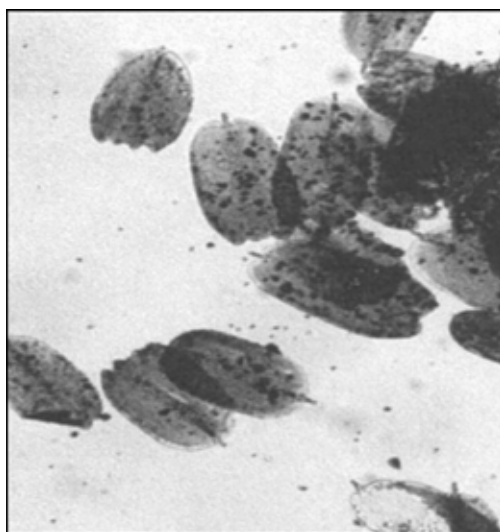


Plate 6.18. Butterfly scales
(Courtesy of Dr. Lillian de las Llagas)

Despite the close association between arthropods and respiratory allergy, there is still no direct evidence available to justify this. Evidence has stemmed from positive skin tests utilizing arthropod extracts, the inability to find other etiological factors to explain respiratory symptoms, and the presence or abundance of arthropods in the immediate environment coinciding with the patients' allergic symptoms. The work of Agbayani et al. in 1989 showed this relationship.

House dust mites (*Dermatophagoides*) have also been implicated as a source of allergens by some investigators. A study on house dust

mites (HDM) by de las Llagas and Abong (2002) on the association between mites and respiratory allergy showed the presence of six species of HDM in dust samples collected from houses of patients with a history of bronchial asthma and allergic rhinitis. These mites are *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Glycyphagus* spp., *Austroglycyphagus*

spp., *Cheyletus malaccensis*, and *Suidasia pontifica* (Plates 6.19–6.22).

Winged insects such as mayflies (Order Ephemeroptera), caddisflies (Order Trichoptera), moths and butterflies (Order Lepidoptera), and aphids (Order Hemiptera), have been observed to be the most common arthropods inducing respiratory allergy.

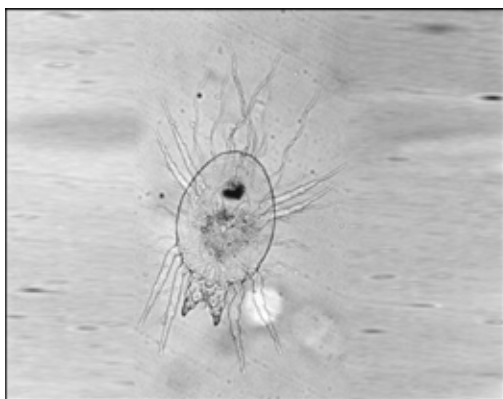


Plate 6.19. Dust mite (*Blomia tropicalis*)
(Courtesy of Dr. Lillian de las Llagas)

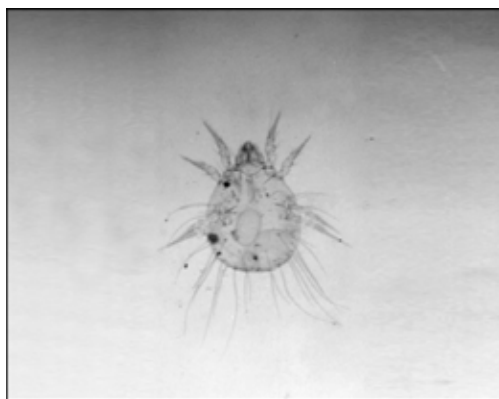


Plate 6.20. Dust mite (*Glycyphagus* sp.)
(Courtesy of Dr. Lillian de las Llagas)

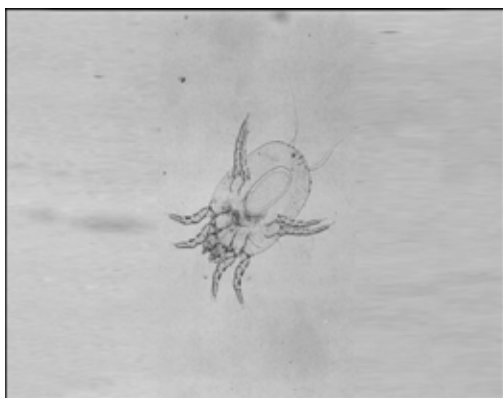


Plate 6.21. Dust mite (*Dermatophagoides pteronyssinus*) (Courtesy of Dr. Lillian de las Llagas)

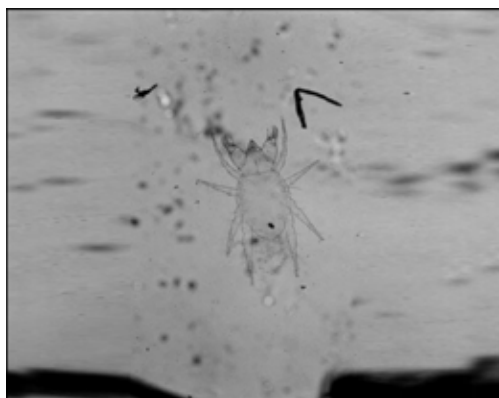


Plate 6.22. Dust mite (*Cheyletus malaccensis*)
(Courtesy of Dr. Lillian de las Llagas)

Ingestants

The feces of cockroaches and the vomitus of non-biting flies are the best examples of harmful ingestants of man. These ingestants are highly contaminated with microorganisms, which are

pathogenic to humans. Diarrheal diseases have long been associated with these arthropods.

Various mites and their eggs, either living or dead, have been found in various parts of the human body, such as the alimentary canal and urinary tract. The presence of mites has been

found to be contributory to various conditions including enteritis, nocturnal enuresis, and hematuria. The evidence, however, is not direct because in many cases, the mites are quite harmless.

The most common mites present on food include species in the genera *Tyrophagus*, *Acarus*, and *Glycyphagus*.

Food and Water Pests

Food and water adulteration/contamination due to insects and mites may be incurred in any of six stages: storage, transport, preparation, processing, packaging, and serving. The insects and mites discussed in this chapter are classified as pests because of the damage done on food and water and the potential risk to humans upon consumption (Table 6.4).

Table 6.4 Arthropods as pests of stored products, food and water sources identified at the Medical Entomology Laboratory, UP-CPH

Arthropods	Food products/water sources/places infested
Chironomid larva or blood worm	Water tanks, hospital faucet
Moth caterpillar	Chocolate candies
Moth pupa	Chocolate bars
Moth adult (<i>Plodia interpunctella</i>)	Chocolate rice crispies
Beetle adult	Hospital bed
Beetle (grain) adult	Raisins
Mites	Pancake mix Bakery products
Fly larvae (<i>Sarcophaga</i> spp.)	Stuffed milkfish
Phorid fly	Bread with sugar coating
Centipede adult (<i>Scolopendra</i> spp.)	Pancit
Cockroach adult (<i>Blattella germanica</i>)	Dimsum from a Chinese restaurant

Myiasis

Myiasis is the infestation or invasion of tissues or organs of humans and animals by dipterous larvae (Plate 6.23). Sometimes,



Plate 6.23. Maggots
(Courtesy of Dr. Lillian de las Llagas)

this occurs accidentally, but for some species, parasitism is necessary. Obligatory myiasis is the condition wherein larvae need a host to complete their development. Facultative myiasis occurs when free-living larvae become parasitic.

Clinically, myiasis may be classified according to the part of the body invaded. Aural, nasal, ophthalmic, cutaneous, and intestinal myiasis have been reported. In the Philippines, myiasis is caused by the following species of fly larvae.

- Obligatory
(*Chrysomya*, *Booponus*, *Stomoxys*, *Lyperosia*)

Animals primarily affected include carabaos, cattle, and other domestic animals.

- Facultative
Phaenicia
Lucilia
- Accidental
Sarcophaga
Pericoma

Identification of Myiasis-Producing Larvae

The identification of fly larvae is important for prevention and control. In forensic medicine,

identification of the species and age of the larvae can help establish the time of death of a victim. Identification is done by examining the morphology of the posterior spiracles and the cephalo-pharyngeal skeletons.

Maggots, the larvae of muscoid diptera, are legless, worm-like and more or less cylindrical. They are usually tapered anteriorly and broad posteriorly. The spiracles are situated on the posterior end (Table 6.5).

Table 6.5. Identifying characteristics of some myiasis-producing larva

Larva	Characteristics
<i>Musca</i>	Posterior spiracles: D-shaped, with spiral slits and a complete peritreme
<i>Chrysomya</i>	Body: with bands of spines
<i>Stomoxys</i>	Posterior spiracles: with a black peritreme and a spiral slit
<i>Lucilia and Phaenicia</i>	Posterior spiracles: lower spiracular slits oriented upward and not horizontal
<i>Sarcophaga</i>	Posterior spiracles: lie in a deep slit, slits not pointing towards the opening of the peritreme Anterior spiracles: with 12 processes, accessory oral hook absent

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Arthropods as Vectors of Disease

Lillian de las Llagas

Arthropods as Transmitters of Pathogenic Agents

Arthropods which are capable of acquiring and transmitting pathogens that cause diseases are called vectors. There are two types of vectors: biological vectors and mechanical or passive vectors. Biological vectors, (e.g., mosquitoes and biting flies), acquire pathogenic agents in the act of blood-feeding. These agents undergo multiplication, propagation, and development inside the arthropod’s body. After some time, the pathogens assume their infective form and are then transmitted from one host to another. Mechanical vectors, on the other hand, transmit pathogens by way of their oral secretions (vomit of flies) and the contaminated external surfaces of their body (feet, wings, etc.). Mechanical vectors serve as mere contaminators; the pathogens do not undergo multiplication or development inside

their bodies. Some vectors (e.g., fleas, beetles, crabs, and copepods) serve as intermediate hosts to some parasites.

Most of the arthropods which are classified as vectors of diseases belong to Class Insecta, subclass Pterygota (winged insects such as mosquitoes, flies, and cockroaches) and Order Acarina (mites and ticks).

Several arthropod-associated diseases in the Philippines are summarized in Table 6.6. The diseases listed have varying degrees of importance. Based on morbidity and mortality, the most important diseases are the mosquito-borne diseases. Others do not rank high among national health care priorities, but they have significant public health implications. Diseases associated with cockroaches and non-biting flies (e.g., diarrhea and amebiasis) are important, although evidence linking diseases to the filthy behavior of these arthropods is purely circumstantial.

Table 6.6. List of arthropod-associated diseases and their corresponding agents and vectors

Disease	Agent	Vector
Malaria	Plasmodium	Mosquito
Filariasis	<i>Wuchereria and Brugia</i>	Mosquito
Dengue/Dengue Hemorrhagic Fever	Dengue virus	Mosquito
<i>Japanese Encephalitis</i>	JE virus	Mosquito
Scrub typhus	<i>Rickettsia</i>	Chigger Mite
Babesiosis	<i>Babesia</i>	Tick
Paragonimiasis	<i>Paragonimus</i>	Crab
Diphyllobothriasis	<i>Diphyllobothrium</i>	Copepod
Dracunculiasis	<i>Dracunculus</i>	Copepod
Hymenolepiasis	<i>Hymenolepis</i>	Flea
Dipylidiasis	<i>Dipylidium</i>	Flea
Raillietiniasis	<i>Raillietina</i>	Flour or Rice Beetle
Amebiasis	<i>Entamoeba</i>	Flies and Cockroaches
Diarrheal Disease	Enteric pathogens	Flies and Cockroaches
Miscellaneous Intestinal Parasitoses	<i>Ascaris, Trichuris</i>	Flies and Cockroaches

The succeeding topics describe the most important vectors of tropical diseases in the Philippines: mosquitoes, flies, and cockroaches.

Mosquitoes

There are two important divisions or tribes of mosquito vectors. The anopheline mosquitoes, consisting of Genus *Anopheles*, which are vectors of human malaria and human filariasis; and the culicine mosquitoes, vectors of dengue, Japanese encephalitis, and human filariasis, which includes the genera *Aedes*, *Culex*, and *Mansonia*. Mosquitoes undergo a complete type of metamorphosis. Fertilized eggs go through four larval stages, develop into the comma-shaped pupae, and then emerge as adults. The immature stages require an aquatic environment, while the adult, an aerial and terrestrial one.

A. Egg

Anophelines lay their boat-shaped eggs individually over the surface of water, each having lateral air floats to keep it buoyant. *Culex* lay their eggs in rafts. Each *Culex* egg is cigar-shaped, and is provided with a corolla at the end. *Mansonia* lay their eggs under leaves of aquatic plants. *Aedes* eggs are laid individually, often in artificial containers, and dry hollows, which become flooded after the rain. These “dry-laid” eggs are able to retain their viability for long periods without water.

B. Larva

Eggs of mosquitoes generally hatch after 2 to 3 days of contact with water. They are about 1 to 1.5 mm long when newly hatched and grow to a full length of about 8 mm. The larva casts its skin four times. The stages between molts are known as instars. The mosquito larva breathes through two openings called spiracles. The spiracles of the anopheline larvae are situated on the eighth abdominal segment so that in order to breathe, the larva rests in a horizontal position at the surface of the water. In culicines, the spiracles are situated at the end of a tubular

organ called the siphon, which extends from the eighth abdominal segment. The culicine larva therefore hangs down from the surface of the water by the tip of the siphon in order to breathe. The *Culex* larva has a long and slender siphon, with many ventral hair tufts. *Aedes* has a short and stout siphon with only one pair of hair tufts. *Mansonia* breathes through a siphon modified for piercing and adhering to stems of aquatic plants.

C. Pupa

This is the non-feeding stage, found on the surface of the water sources. The pupa is mobile and is able to dive rapidly when disturbed. It breathes through a pair of respiratory trumpets. Culicine pupae have longer trumpets than anophelines.

D. Adult

Generally, the wings of anophelines have dark and pale areas, whereas culicines have unpatterned wings. Another visual distinction is that, at rest, the body of an anopheline mosquito forms an angle nearly vertical with the surface (i.e., the head, thorax, and abdomen are in a straight line). The culicine mosquito, on the other hand, lies almost parallel to the surface, sometimes appearing as “hump-backed.”

The abdominal tip is pointed in the female *Aedes*, and blunt in *Culex*. *Mansonia* has speckled legs with asymmetrical brown, yellow, and gold scales.

Palpi of female *Anopheles* are as long as the proboscis. Palpi of its males are club-shaped, each with rounded scutellum. Palpi of female culicines are not as long as the proboscis (usually a quarter of the proboscis); male culicine palpi are not clubbed, and the scutellum is trilobed.

E. Mosquito Bionomics

Bionomics deals with the relationship between a species and its environment. An understanding of mosquito bionomics is important in the epidemiology of mosquito borne diseases, and in planning methods of

mosquito control. The environment consists of the climate, the water habitat of immature stages, and the hosts for the adults. The environment of immature and adult mosquitoes is interdependent, because the female mosquito must have access to water for egg-laying. The adult environment is largely aerial and terrestrial, the former for mating and dispersal, and the latter for feeding and resting.

The Environment and Habits of the Adult Mosquito

A. Mating

Mating usually occurs within 24 to 48 hours after emergence. In some species, the males form a swarm, usually at dawn or in the evening. Females entering the swarm are seized, and the resulting pairs drop out of the swarm. Insemination then follows.

B. Dispersal

The male is a much weaker flyer than the female. Most mosquitoes fly within a range of 1 to 2 km. Strong winds carry mosquitoes along greater distances.

C. Biting Habits

Host seeking and feeding generally take place in a warm, humid environment. Biting hours vary from one species to another. *Culex*, *Mansonia*, and *Anopheles* prefer to bite at night while *Aedes* during daytime. Mosquitoes which feed while inside human dwellings are described as endophagic, while those that feed outdoors are called exophagic.

D. Resting Habits

After feeding, adult mosquitoes may rest inside dwellings, referred to as endophily or may rest outdoors, referred to as exophily.

E. Host Preference

Mosquitoes that feed on humans are called anthrophilic, whereas those that feed on animals are zoophilic.

F. Seasonal Prevalence

In tropical countries, such as the Philippines, where there are no extreme fluctuations in temperature and humidity, rainfall is the most important factor affecting the mosquito population. The rise and fall of the mosquito density, called seasonal fluctuation, is dependent on the availability of suitable aquatic environments, which can support the multiplication of the mosquito.

The Philippines has four types of climate based on monthly rainfall. Type I areas have two pronounced seasons, dry and wet; Type II areas have no dry season, but with a very pronounced rainfall; Type III areas have seasons not very pronounced; and Type IV, where rainfall is more or less evenly distributed throughout the year.

With these types of climate, it is possible to expect the following:

- In Type I areas, it is possible to have two density peaks: one during intermittent rains and the other before the onset of heavy rains.
- In Type II areas, more breeding grounds are expected
- In Types III and IV areas, there will be no peak months; thus, mosquito populations are maintained at certain levels.

G. Extrinsic Incubation Period and Longevity

The climate in which the mosquito lives dictates its capability for disease transmission. The climate influences the rate of development of the parasite within the vector, and the longevity of the mosquito.

The period between the mosquito's infected blood meal and its transmission of the infective agent in a subsequent feeding is called the extrinsic incubation period. It varies in length in response to the temperature of the host mosquito's environment. For example, the development of the malaria parasite, *Plasmodium* is retarded at 19°C down to 15°C

and below, but completed at 20 to 30°C. Also, the growth of the filarial parasite *Wuchereria* in *Culex quinquefasciatus* is inhibited at mean temperatures below 24°C and above 34°C.

Temperature and humidity affect the survival of mosquitoes. At extremely high or low humidities, mosquitoes are unable to regulate their water loss. They thrive best at 70 to 80% relative humidity and at a temperature of 20 to 30°C.

Major Mosquito-Borne Diseases

A. Malaria

The vectors of malaria in the Philippines include: *Anopheles flavirostris*, the primary vector of malaria; *Anopheles litoralis* and *Anopheles balabacensis*. *Anopheles flavirostris* is found in the entire national territory, except in areas with elevations of more than 4,000 ft. *Anopheles litoralis* has been described in Basilan, various Luzon provinces, Southern Samar, Sulu, Surigao, and Zamboanga. *Anopheles balabacensis* has been reported only in Palawan.

Morphological Characteristics, Breeding Places, and Habits of Vectors of Malaria

1. *Anopheles flavirostris*

This is the most important vector of malaria in the Philippines. It is a small- to medium-sized mosquito, measuring 2 to 6 mm in length. It has a proboscis with a pale golden patch that is usually confined to its apical half. The basal third of its costal vein is usually dark or has a single pale spot.

An. flavirostris usually breeds in slow flowing, clear, partially shaded streams with vegetation. It also breeds in foothills and in wells. During the rainy season, it is possible to collect the larvae from rice fields and trapped waters.

This mosquito is widespread in distribution. It has been reported to be endophagic, endophilic, and anthrophilic. Recent observations by field entomologists showed that female mosquitoes prefer to rest

outside human dwellings. This may indicate that *An. flavirostris* exhibits certain degrees of exophily and exophagy. These observations deserve serious attention, as the current indoor residual spraying of insecticide may no longer be effective.

Deviations in the characteristics of this mosquito have been observed, and this may disqualify the claim that *Anopheles flavirostris* is made up of one or two species.

2. *Anopheles litoralis*

This small- to medium-sized mosquito is a secondary vector (supplementary role in transmission but would be unable to maintain an epidemic in the absence of primary vector) of malaria. It has palps with three pale bands: the pale band at the tip is broad, the next is narrow, and the third very narrow. Its legs are speckled, the hind tarsi possessing apical distinct narrow pale bands. They prefer to breed in water with a salinity of 2.5 to 3.0%.

3. *Anopheles balbacensis*

This is also a secondary vector of malaria. It is a small- to medium-sized mosquito having palps with narrow pale bands. It has a dark proboscis and wings with multiple dark spots. It also has speckled legs, with wide bands on the tibiotarsal joint of the hind legs. This mosquito breeds in clear ponds and pools in deep forests and jungles.

B. Filariasis

The vectors of Bancroftian filariasis in the Philippines include *Aedes poecilus*, which breeds in abaca-raising areas, and *Anopheles flavirostris*, which breeds in clear mountain streams. The vectors of Malayan filariasis in the Philippines include *Mansonia bonneae* and *Mansonia uniformis*, which breed in swampy and forested areas.

Aedes poecilus has been reported in the Bicol region, Masbate, areas of Mindanao, Mindoro, Quezon and Sulu. *Anopheles flavirostris* has been shown to transmit the parasite in Mt. Province

(Bontoc), Palawan and Sulu. *Mansonia* has been found in Agusan del Sur, Eastern Samar, Palawan, and Sulu.

Morphological Characteristics, Breeding Places, and Habits of Vectors at Filariasis

1. *Aedes poecilus*

This mosquito is associated with Bancroftian filariasis. It breeds in the axils of plants like abaca (*Musa textiles*), banana (*Musa sapientum*), pandanus, gabi (*Colocasia esculentum*), and *biga* (*Alocasia macrorrhiza*).

The adult *Aedes poecilus* has scutellar scales that are mostly broad and white. The dark scales are found on the mid-lobe and form a distinct dark central patch. A variable number of white scales are also present at the base of the first four tarsal segments.

This mosquito is a nocturnal feeder. However, it is possible to find it seeking a blood meal during the day. It is highly anthrophilic but it may feed on animals like birds, bovids, and dogs. The highest density of these mosquitoes is observed from 10 p.m. to 12 a.m., which coincides with *W. bancrofti* periodicity. The density of these mosquitoes is also related to rainfall patterns in endemic areas. This mosquito is endophilic and partially exophilic.

2. *Mansonia*

A vector of the Malayan type of filariasis, *Ma. bonneoe*, is a forest swamp mosquito. It prefers fresh water swamps with an extensive growth of giant pandanus. *Ma. uniformis* also breeds in swamps containing other aquatic plants.

These mosquitoes are exophagic and exophilic. The peak of biting is observed at 1:00 a.m. to 2:00 a.m.

The population density of *Aedes* mosquitoes and *Mansonia* is related to rainfall patterns.

3. Adult *Mansonia*

It is a medium-sized, robust-built mosquito, usually light to dark brown, or light yellow

to golden in color. Its legs have many pale markings, and its wings have white and dark broad scales, many of which are asymmetrical.

C. Dengue/Dengue Hemorrhagic Fever

The vectors of dengue in the Philippines include *Aedes aegypti*, which is associated with urban dengue, and *Aedes albopictus*, which is associated with rural dengue. There is a widespread distribution of these vectors in the Philippines.

Morphological Characteristics, Breeding Places, and Habits of Vectors of Dengue

1. *Aedes aegypti*

This is primarily known as the “tiger mosquito.” It is black in color, and small to medium in size. It has characteristic lyre-shaped, silvery markings on its mesonotum. The fore- and mid-pairs of legs have white narrow bands at the base of the tarsi. The hind pair of legs has five broad white bands, hence the name “tiger mosquito” (Plate 6.24).

This mosquito breeds in clear water collecting in indoor and outdoor containers such as old tires, vases, jars, and bottles.



Plate 6.24. *Aedes aegypti* mosquito
(Courtesy of Dr. Lillian de las Llagas)

2. *Aedes albopictus*

The most important diagnostic characteristic of this mosquito is the presence of a single, longitudinal, silvery stripe on the mesonotum. This mosquito breeds in clear water collecting in indoor and outdoor containers such as bamboo stumps, empty coconut shells, some artificial containers, and tree holes. It is not unusual, therefore, to see both *Aedes* species sharing a common habitat.

D. *Japanese Encephalitis (JE)*

The proven vector of Japanese encephalitis in the Philippines is *Culex tritaeniorhynchus*. Potential vectors include *Culex vishnui*, *Culex gelidus*, and *Culex fuscocephalus*. The vectors are widely distributed in ricefields. Most cases of JE are from Luzon, particularly from Nueva Ecija.

Morphological Characteristics, Breeding Places and Habits of Vectors of Japanese Encephalitis

1. *Culex tritaeniorhynchus*

This is a small mosquito. The mesonotum is uniformly covered with dense, very small, brown to dark brown scales, which are curved and narrow. Its proboscis has a pale band. This mosquito is usually associated with rice fields.

Activity is greatest from 6:00 p.m. to 7:00 p.m. The mosquito feeds on man and animals, specifically pigs. Pigs serve as amplifying hosts.

Flies

There are different species of non-bloodsucking flies that are commonly encountered in our environment. These flies that coexist with humans over an extended period of time are described as synanthropic species. The most common representative is the common housefly (*Musca domestica*).

Synanthropic flies are associated with gastrointestinal diseases such as amebiasis, salmonellosis, and shigellosis. This association stems from their filthy habits; they feed on human and animal excreta, then freely feed on

human food. Anatomically, these flies are well adapted to carry and disseminate pathogenic agents because of the following structures:

1. Sponging mouthparts. The expanded labellum has hairs that are capable of sweeping or picking up the agents.
2. Manner of ingesting food. A drop of saliva is regurgitated in the process and this contaminates the food.
3. Hairy body and appendages.
4. Foot pads. These are also contributory to their pathogen-carrying potential because of their sticky secretion.

Pathogenic agents acquired and carried by these flies include *Ascaris*, *Trichuris* and hookworm ova. The extent of disease transmission by adult flies under natural conditions is difficult to determine. The larvae of flies may also affect humans. These larvae or maggots invade living tissues, producing a condition called myiasis.

A. *Musca domestica* (The common housefly)

This fly is dark gray in color and measures about 6 to 9 mm in length. It has four conspicuous longitudinal black bands or stripes on its thorax. The arista has dorsal and ventral hairs. The wing venation is characterized by Vein 4 (V4) bending sharply at the end of Vein 3 (V3). The two veins are therefore very close at the edge of the wing.

The eggs of the common housefly are laid in masses of about 75 to 150 eggs. A single female is able to lay as many as 21 batches within a month after emergence. Hatching takes place in about 20 to 24 hours under warm conditions, and the resulting legless, headless, and eyeless larva, or the maggot, undergoes three stages of development. The maggot completes its development in about 5 to 9 days then it migrates to drier habitats and changes into a pupa. The pupal state requires 4 to 7 days before an adult emerges, making a total of about 10 to 17 days of development from egg to adult.

Other species of synantrophic flies include: *Sarcophaga* (flesh fly), *Calliphora* (blue-bottle fly), *Lucilia* (green-bottle fly), *Muscina* (non-biting stable fly), and *Fannia* (latrine fly).

B. *Sarcophaga*

The adult fly measures 11 to 15 mm long and is gray in color. It has three prominent black longitudinal stripes on the dorsum of its thorax. The abdomen is distinctly marked with squarish dark patches on a gray background, giving it a “chess-board” appearance. Adults do not lay eggs. Larval development is about 3 to 4 days, while stage lasts about 7 to 14 days.

C. *Calliphora*

The face or genae of the adult is covered with yellow hairs. The fly is bluish in color, and its thoracic hairs are well-developed. The life cycle of this fly requires 16 to 35 days, usually 22 days.

D. *Lucilia*

This fly is greenish in color and has white genae. Its thoracic bristles are well developed, and there are two pairs of acrostichal bristles on its mesothorax. The life cycle of this fly is similar to that of *Calliphora*. A very similar species is *Phaenicia* (bronze-bottle fly).

E. *Muscina*

This fly is slightly larger and more robust than the housefly. It is dark gray to almost black in color. It has four longitudinal black bands on the thorax, and its arista bears setae. Vein 4 (V4) is not much angled, and is clearly separated from Vein 3 (V3) at the wing margin.

F. *Fannia*

This fly resembles *Musca domestica* very closely but it is smaller and more slender. The arista is bare; V3 and V4 are broadly open.

Cockroaches

Cockroaches, like non-blood sucking flies, are also carriers of some pathogenic organisms. The best example of their filthy habits is feeding on human feces and then on human food.

At least 16 species of cockroaches are considered carriers of pathogenic agents. The three most common are *Periplaneta Americana*, *Blatella germanica*, and *Blatta orientalis*. Cockroaches are nocturnally active, but they may be seen crawling at daytime. Cockroaches are much bigger than flies and thus enabling them to carry more pathogens. Transmission of pathogens is facilitated by their hairy chewing mouthparts, which enable them to pick up pathogens easily, and their habit of dropping their feces while walking or feeding. A study conducted in the University of the Philippines Manila-College of Public Health in 1981 recovered the following parasites and pathogens from *Periplaneta americana*: *Ascaris*, *Trichuris*, and parasites under Family Thelastomatidae and Superfamilies Spiruroidea and Tylenchoidea. Other pathogens include *Proteus*, *Escherichia*, *Salmonella*, and *Citrobacter*.

The extent of disease transmission by cockroaches under natural conditions is not clearly known.

A. *Periplaneta americana* (American cockroach)

This cockroach is chestnut brown to dark reddish-brown in color. It is the largest species among the three most commonly encountered domestic cockroaches. It measures up to 40 mm in length, and both male and female adults have fully developed wings. The female, in her lifetime, lays about 50 egg capsules or ootheca, each containing about 15 eggs. The length of the life cycle is from 6 months to a year (Plate 6.25).



Plate 6.25. American cockroach (*Periplaneta americana*) (Courtesy of Dr. Lilian de las Llagas)

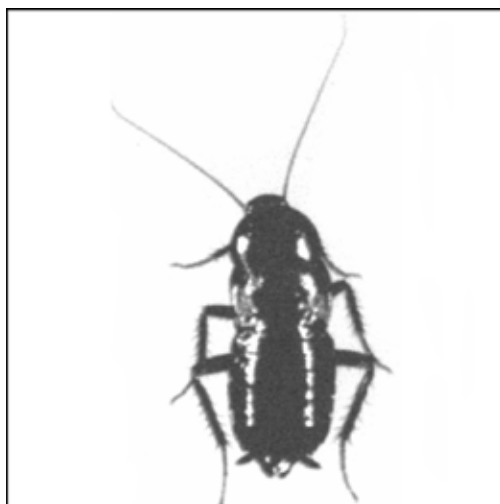


Plate 6.27. Oriental cockroach (*Blatta orientalis*) (Courtesy of Dr. Lilian de las Llagas)

B. *Blatella germanica* (German cockroach)

The German cockroach measures 10 to 15 mm in length. It is pale yellowish-brown in color. It has two prominent longitudinal dark bands on its pronotum. The female carries the ootheca, which protrudes from the tip of the abdomen, until hatching time. Its life cycle takes from 2 to 3 months (Plate 6.26).

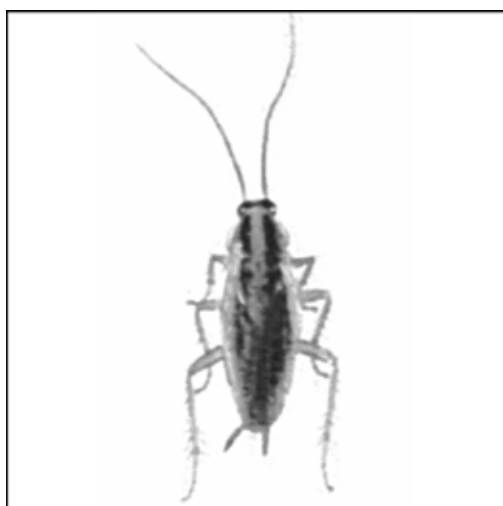


Plate 6.26. German cockroach (*Blatella germanica*) (Courtesy of Dr. Lilian de las Llagas)

The Oriental cockroach measures 22 to 27 mm long. It is dark brown to black in color, and both sexes show wings that are very short. The length of its life cycle is 12 months (Plate 6.27).

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Medical Malacology

Lydia R. Leonardo

Mollusks are the second most numerous animals on earth. They include snails, slugs, clams, oysters, chitons, squids, octopods, and nautili. One class, the Gastropoda, contains groups that are directly injurious to man, or are essential intermediate hosts of helminth parasites. The poison cone shells have stinging apparatus that are capable of discharging highly toxic substances. Trematodes require specific species of mollusk as their intermediate hosts. The strongyliid nematodes use the ordinary garden snails and slugs as intermediate hosts to complete their life cycle. Some 350 snail species are estimated to be of possible medical or veterinary importance because of their involvement in the life cycle of human parasites.

Medical malacology deals with the biology, ecology, and taxonomy of snail groups that are of medical and public health importance. This fundamental knowledge is an important basis for designing control and prevention programs for helminth parasites with snail intermediate hosts. This section provides a list of snails of medical importance, including their taxonomic classification, their biology, and ecology. The effect of parasites on snail intermediate host is also given. Lastly, snail control in relation to the control and prevention program for schistosomiasis is discussed.

Taxonomy of Snails of Medical Importance

Mollusks are divided into six classes, namely: the Class Monoplacophora represented by only one living genus *Neopilina*, with a few species; the Class Amphineura, the chitons; the Class Gastropoda, the most numerous, represented by snails and slugs; the Class Cephalopoda, the squids, cuttlefish, the octopods, and the nautili; the Class Scaphopoda, the marine tooth or tusk shells; and the Class Pelecypoda, the bivalves.

The medically important snails belong to Class Gastropoda. They are distributed into two subclasses, namely, Prosobranchiata and Pulmonata. The prosobranchs are operculate snails with well-formed shells and gills. They have a snout-like head-foot, one pair of retractile tentacles, and one pair of eyes. The sexes are separate, and eggs are usually laid in capsules. Some are ovoviviparous. On the other hand, pulmonates are air-breathing snails and slugs with shells that are reduced or even absent, and with a head-foot that bears two pairs of tentacles. All pulmonates are monoecious, and most are oviparous.

The distribution of the medically important snails in the two subclasses is as follows:

A. Subclass Prosobranchiata

1. Order Neogastropoda

Family Conidae – species *Conus*

2. Order Mesogastropoda

- a. Family Thiariidae – *Thiara* spp. (intermediate host of *Paragonimus westermani*, *Metagonimus yokogawai*, and other heterophyid flukes in the Orient)
- b. Family Pleuroceridae – *Semisulcospira* spp. (intermediate host of *Paragonimus westermani* in the Orient) and *Goniobasis plicifera silicula* (intermediate host of *Troglorema salmincola* in the Pacific Northwest in the United States)
- c. Family Potamididae – *Pironella conica*, *Cerithidia cingulata*, and *Pyrazus ebeninus* (intermediate hosts of *Heterophyes heterophyes* and hosts of cercariae causing schistosome dermatitis)

- d. Family Pilidae – *Pila* spp. (intermediate host of *Parastrongylus cantonensis* and *Echinostoma ilocanum*)
- e. Family Synceridae – *Syncera luteola* (intermediate host of *Paragonimus iloktsuenensis* in rodents in China)
- f. Family Hydrobiidae
 - i. Subfamily Hydrobiinae – *Oncomelania* spp. (intermediate host of *Schistosoma japonicum*) and *Pomatiopsis lapidaria* (intermediate host of *Paragonimus kellicoti*)
 - ii. Subfamily Buliminae (syn. Bythiniinae) – *Parafossarulus* spp. and *Bulinus* spp. syn. *Bythinia* and *Bithinia* (intermediate hosts of *Opisthorchis felineus*, *Clonorchis sinensis*, *Metagonimus yokogawai*, and *Echinochasmus perfoliatus*)

B. Subclass Pulmonata

1. Order Basommatophora

- a. Family Lymnaeidae – *Lymnaea*, *Fossaria*, *Pseudosuccinea*, *Radix*, *Stagnicola* (first intermediate hosts of *Fasciola hepatica*, *Fasciola gigantica*, several species of *Echinostoma*, *Plagiorchis*, and freshwater dermatitis-producing schistosome cercariae)
- b. Family Planorbidae
 - i. Subfamily Planorbinae – *Biomphalaria* spp. (intermediate host of *Schistosoma mansoni* in Africa and Near East and in tropical America) and *Gyraulus* spp. (intermediate host of *Echinostoma ilocanum* in the Orient)
 - ii. Subfamily Helisomatinae – *Helisoma* spp. (intermediate host of *Echinostoma revolutum*

in the United States and Mexico) and *Planorbarius metidjensis* (intermediate host of *Schistosoma haematobium* in Portugal and Morocco)

- iii. Subfamily Segmentininae – *Segmentina* spp. and *Hippeutis* spp. (both intermediate hosts of *Fasciolopsis buski* and *Echinostoma ilocanum* in the Orient)
- iv. Subfamily Bulininae – *Bulinus* spp. (intermediate host of *Schistosoma haematobium* in Africa, Near East, Middle East) and *Indoplanorbis exustus* (intermediate host of *Schistosoma spindale*, *S. nasale* in India, Malaysia, and Sumatra)

- c. Family Ancyliidae – *Ferrissia tenuis* (intermediate host of *Schistosoma haematobium* in India)
- d. Family Physidae – *Physa* spp. (intermediate host of *Echinostoma revolutum* in the Orient and schistosome cercariae producing dermatitis from freshwater and marine shoreline snails)

2. Order Stylommatophora

- a. Family Achatinidae – *Achatina fulica*, also known as giant African land snail (intermediate host of *Parastrongylus cantonensis*)
- b. Family Helicellidae – *Helicella candidula* (intermediate host of *Dicrocoelium dendriticum* in Europe and Western Asia)
- c. Family Cionellidae – *Cionella lubrica* (intermediate host of *D. dendriticum* in the United States)
- d. Family Limacidae – common slugs *Limax* and *Deroceras* (intermediate hosts of lungworms of domestic mammals and experimentally of *Parastrongylus cantonensis*)

3. Order *Systellommatophora*

- a. Family Veronicellidae – several species in South Pacific Islands, China Sea area, Australia, and Cuba, and are hosts of *Parastrongylus cantonensis*; species in American tropical areas are hosts of *Parastrongylus costaricensis*.

The specific identity of gastropod intermediate host is important, especially in appreciating the susceptibility and non-susceptibility of snail hosts and various aspects of host-parasite relationships. Malek suggested that the only way to understand issues in taxonomic identification is to be aware that species vary in space and time; hence, intraspecific variations as evidenced by discrepancies in susceptibility to infection is observed between certain local races of snails and races of parasites.

Snail identification based on shell features and soft parts has been fraught with problems, since the shell varies with age, the type of habitat, and even the quality of water in aquatic habitat. In addition to morphology, new approaches to resolve issues in taxonomy and systematics have involved cytological studies, biochemical studies, serological methods, and molecular means. These studies have focused mainly on snail intermediate hosts of schistosomes found in endemic countries in Asia, South America, and Africa. For the main Asian schistosome, the snail includes various species of *Oncomelania*.

In the past, the amphibious snail intermediate host of *Schistosoma japonicum* was considered to be one species, *Oncomelania hupensis*, with six subspecies with separate geographic distribution. These are *O. h. hupensis* from mainland China; *O. h. formosana* and *O. h. chiui* from Taiwan; *O. h. nosophora* from Japan; *O. h. quadrasi* from the Philippines; and *O. h. lindoensis* from Sulawesi, Indonesia. Results of biochemical, antigenic and genetic studies suggest that *O. hupensis*, *O. formosana*, *O. nosophora*, *O. quadrasi*, and *O. lindoensis* should be elevated to independent species

status. Further and more distinct classification of *Oncomelania* spp. will require advanced genetic, morphological, and biochemical studies. Snail intermediate hosts of *S. mansoni* and *S. haematobium* are also reported to possess physiological differences affecting host-parasite relationship. Similarly, the alpha race and gamma race differ from one another in morphology of the x-chromosome of *Neotricula aperta*.

Efforts to clarify the taxonomy and phylogeny of the genus *Schistosoma* and its snail intermediate host, one of which is the genus *Oncomelania*, continue. In the Philippines, research on the proper classification of *S. japonicum* and *O. quadrasi* are sporadic and sketchy.

Distribution of Snail Intermediate Hosts

Snail intermediate hosts are found in almost all types of habitats. These range from small temporary ponds and streams to large lakes and rivers. There are important factors that influence these habitats, such as the amount of sunlight that penetrates the water, food availability, strength of the current, nature of the substratum, ionic composition of the water, extent of growth of aquatic weeds, and the presence or absence of parasites and predators. Ponds, pools, swamps, ditches, and canals are usually shallow enough for the snails, and allow sunlight, favoring photosynthesis of phytoplankton and plant organisms. Water currents and other movements may aerate water, but could also detach snails from their anchorage. Members of families Pleuroceridae and Thiaridae are able to hold up in swift, but not torrential water, better than members of the family Hydrobiidae. Buliniids are stronger than the biomphaliariids when it comes to withstanding water currents. Larger snails are better anchored compared to smaller snails, while those snails with larger aperture are observed to fare better than those with smaller aperture.

Temperature and altitude affect snail habitats by changing the rate of photosynthesis and the rate of decomposition, as well as the rate of reproduction of the resident snails. Permanence and stability of the habitat are critical factors affecting the presence of snails. Water levels affect the balance in the ecosystem, particularly those involving the producers, consumers, and reducers. Small- or medium-sized habitats are less stable than the bigger ones. Snails naturally prefer to build large populations in permanent habitats where they can reproduce and establish more secure colonies. Snails that find themselves in non-permanent habitats take advantage of favorable periods by reproducing rapidly. They also resort to estivation to survive adverse conditions of drought.

Aside from physico-chemical factors, there are biological factors that affect snail distribution in a potential habitat. Aquatic vegetation can serve as anchorage, and microflora provide food sources. Bacteria and fungi are pathogens that may be detrimental to snails. Predators such as insects, crabs, crayfishes, other snails, fishes, amphibians, birds, and mammals can feed on the snails. Lastly, snails are susceptible to parasites like digenetic trematodes and nematodes. Snail distribution is usually patchy; therefore, habitats should be examined at different sites. Seasonal variations also affect snail densities.

Aquatic snail hosts of schistosomes inhabit shallow water near the margins of lakes, ponds, marshes, streams, and irrigation canals. They are found creeping on water plants and mud that is rich in decaying organic matter, or on rocks, stones or hard objects covered with algae, or on various types of debris. They abound in waters where water plants thrive, and where the water is moderately polluted with organic matter, such as feces and urine, as is often the case near human habitations.

Vegetation is an important component of the habitat since this provides not only a food source, but also substrates for oviposition and

protection from increased water velocities and predators, such as fish and birds, and maintains a suitable temperature and humidity. Aquatic species die when they get trapped on dry land during the dry season. Amphibious species like the oncomelanids can survive dessication by burying themselves in mud while sealing their apertures with their operculum. They can withstand longer periods of drought in the temperate zone than in the tropics. Oncomelanids are found both in and out of the water in moist areas, such as poorly tilled rice fields, sluggish streams, secondary and tertiary canals of irrigation systems, swamps, and roadside ditches.

Snail-Parasite Interaction

Host specificity is noted to be very high in the choice of snail intermediate hosts by the digenetic parasites. Out there in the aquatic snail habitat, a schistosome miracidium can most likely penetrate other species of snails, but its biochemical adaptation to its compatible snail species will determine its fate in the tissues of the snails. In a compatible snail species, the miracidium is able to develop with the slightest of problems into the cercariae. There might be slight or restricted encapsulation, which causes little damage to the parasite. In other species however, they are walled off and unable to develop further as a result of the strong host reaction brought about by the innate cellular defense mechanisms. These capsules that trap the parasites eventually result in the latter's destruction.

While the chemical basis for the death of the parasites in incompatible snail hosts remains unclear, encapsulation by leukocytes and/or fibroblasts resulting in death is the simplest way of explaining the most effective form of innate resistance in mollusks against incompatible trematode larvae. This shows that susceptibility or resistance of snail to infection is a hereditary character.

Cross et al. proposed that the snail-trematode compatibility is a highly specific relationship often at the population or strain level for both participants. In the course of millions of years of selection and adaptation, the authors proposed that the vector-parasite compatibility has reached its optimum condition, particularly between the local species of *Oncomelania* and the local strain of *S. japonicum*.

After the miracidium settles in a compatible snail host and starts the intramolluscan development, the pressure effects manifest as congestion of the blood sinuses due to migration and maturation of the sporocysts. Other general effects include toxic effects that may lead to destructive changes in organs, particularly the digestive glands, starvation as nutrients are drained by the parasites, and tissue reaction particularly noted as marked generalized proliferative tissue reaction around dead and trapped cercariae.

The most affected organ, the digestive gland or the hepatopancreas, shows radical histopathological modifications such as displacement of tubules and loss of branched nature, erosion of the tubules' epithelium, rise in the number of cytoplasmic vacuoles, overall destruction of gland epithelium and neighboring tissues, and significant reduction in the size of the glands.

At the cellular level, marked changes are noted, such as: cristolysis and reduction in size and number of mitochondria; slight atrophy of the Golgi apparatus in the secretory cells of the epithelium and digestive glands; irregular outline of secretion granules; myelin figures and electron dense material filling up vacuoles; and connective tissue matrix becoming more electron dense and filled, accumulating collagen-like fibers.

Marked alterations in the biochemistry of the parasitized snails are shown by the decreased level of host glycogen and blood proteins, including fluctuations in lipid content suggesting that food reserves are being

consumed by the parasites. There is an overall reduction in proteins and free amino acids, especially the methionine and heme-containing moiety of hemoglobin, which is eaten up by the parasites. Furthermore, there is an increase in the activities of acid and alkaline phosphatases resulting in increased intracellular activities, and in exchange of polysaccharides between host and parasite. Significant reduction in glycogen reserve weakens the host tolerance to anaerobic conditions.

The presence of parasites affects growth, fecundity, life span, heart rate, respiration, and thermal tolerance of the snail host. Growth rate is reduced among infected snails, especially among younger snails. Reduction in size and degeneration of the albumen gland result in lowered egg production. Ohmae et al. in 2003 reported that oogenesis was abnormal in infected snails, as shown by fewer eggs laid and poor hatching ability. Declining heart rate and oxygen uptake have an effect on the metabolic rate. Other physiological changes include lowered maximum thermal tolerance limit and hemolymph osmolarity. Snails with heavy infection have been shown to have higher mortality. In general, infected snails are less mobile and migrate more slowly.

Snail Control

Snail control is an integral component in the control and prevention of digenean parasites, especially schistosomes. Elimination of schistosomiasis through chemotherapy alone is difficult. Japan is credited with having eliminated schistosomiasis in the absence of a well-accepted drug of choice (i.e., praziquantel), mainly relying on measures that targeted the snail intermediate host with considerable success.

Physical control by handpicking may be suitable for large terrestrial snails, but in the early phase of schistosomiasis control, the Japanese government resorted to massive collection of *O. nosophora* by residents in

endemic areas, providing various incentives to promote this campaign. This method may be hard to implement in the Philippines because of the extreme difficulty in locating and collecting the local species of snail intermediate host, which are usually found underneath leaf litter and mud.

Chemical control using molluscicides can wipe out huge populations of snails and should be done using appropriate strategies. Chemical molluscicides include potassium aluminum sulfate, calcium arsenate, NaPCP (sodium pentachlorophenate), Yuramin (3,5-dibromo-4-hydroxy-4-nitroazobenzene), B-2 (sodium 2,5-dichloro-4-bromophenol), and niclosamide (2',5-dichloro-4-nitrosalicylanilide). Niclosamide has been proven to be the most versatile and most effective of these synthetic molluscicides, and has become the molluscicide of choice. In recent years, however, the use of niclosamide has been restricted following claims of its deleterious effect on the environment and non-target organisms. Plant derivatives have been shown to have molluscicidal properties. Endod fruits (*Phytolacca dodecandra*) are used in Africa to kill snail intermediate hosts of *S. mansoni* and *S. haematobium*. In the Philippines, *Croton tiglium*, *Jatropha curcas*, and *Entada phaseoloides* have been proven to have promising molluscicidal efficacies.

When resources are limited and snail colonies are confined to limited areas, focal mollusciciding is effective. Area-wide mollusciciding is recommended in endemic areas where transmission is spread over a watershed or an irrigation system. Repeated applications of molluscicides are needed and must be accompanied by vegetation clearing to make sure that repopulation of snails is prevented. The use of chemical molluscicide has been banned in the Philippines in compliance with a widespread campaign because of its harmful effects on non-target organisms and accumulation in the environment.

Ecological control focuses on the alteration of snail habitats to reduce survival of the snails and to slow down or prevent their breeding. This includes radical modification of the environment to destroy snail habitats and their residents. It may be as extreme as removal of water by drainage, and proper water management in irrigation systems that may involve stream channelization, seepage control, and construction of diversion and intercepting channels. This can be very expensive and will require participation of the local irrigation agency.

Removal of shade or shelter from the sun by clearing of vegetation exposes the snails with deleterious effects. Although this method produces favorable results, sustainability is a major problem since this has to be done regularly and is labor-intensive. Cementing linings of irrigation canals or making them more perpendicular prevents snails from breeding on the banks or margins of streams and irrigation canals. This was one of Japan's ways of controlling *O. nosophora*, and to date, this is seen also as evidence of better agricultural management.

Velocity of water can be accelerated to dislodge snails by proper grading and cleaning of the stream bed and removal of debris. If the area cannot be drained, the depth of the water may be increased rendering it uninhabitable to snails. Snail habitats may be simply covered with landfill.

Japan's success in eliminating snails in Kurume can be attributed to conversion of the marshy lands into extensive golf courses and orchards. Constant monitoring and surveillance of the once endemic area has consistently failed to yield *O. nosophora*.

Ecological control methods can be incorporated into agricultural programs. Results can be permanent if adequately maintained, as shown by the experience in Kurume, Japan. Increased agricultural productivity is assured. The activities can be locally initiated and do

not require foreign exchange, unlike the use of chemical molluscicides.

Corollary to ecological control is proper rice cultivation, which brings about environmental changes and increased productivity. With rice fields serving as important snail habitats, measures such as deep plowing that turns over the soil and buries the snails, harrowing that removes the weeds which provide cover, spacing that exposes them to sunlight, and weeding that removes vegetation, are surefire ways of destroying the snails. Pesticides used by farmers may even be molluscicidal. The stoppage of flow of irrigation water between harvesting and planting can certainly interrupt breeding. Drainage makes sure that waterlogged areas are prevented from becoming transmission sites. There have been efforts in some endemic areas in the Philippines to coordinate snail control with the local agriculture agency, especially where farming methods and irrigation are involved.

In evaluating the effectiveness of the snail control program, certain parameters should be measured, such as reduction in size of area inhabited by snail population, reduction in snail density, change in population structure, and mortality or percentage of dead snails as a result of mollusciciding. Monitoring should include regular checks of snail density and population structure.

The Future of Snail Control

Experience in many endemic countries shows that snail control is an integral part in any program to eliminate snail-borne parasitic diseases, foremost of which is schistosomiasis. Since the discovery of praziquantel, control programs have focused mainly on control of morbidity by chemotherapy. Japan eliminated schistosomiasis even before the advent of praziquantel primarily through snail control. To date, *O. nosophora* still thrives in rice fields and other habitats in the Kofu Basin but has been eradicated in Kurume along the Chikugo River.

The successful elimination of schistosomiasis in Japan emphasizes the fact that there can be snails even without the disease, and that the snails can be eliminated by radical transformation of the environment resulting in widespread destruction of the snail habitats. Molluscicides, may result in large scale mortality of snails but may not be enough to kill them all. Altering the environment to make it uninhabitable to snails is effective, but the cost and effect on the environment are still uncertain.

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Diagnostic Parasitology

Examination of Stool and Body Fluids

Winifreda U. de Leon

Laboratory Diagnosis

Most parasitic diseases cannot be established based on clinical signs and symptoms alone. Confirmation of a suspected parasitic condition generally depends on the result of proper laboratory examination. Correct diagnosis can likewise provide prompt treatment thus preventing possible complications that may arise. Correct diagnosis of parasitic infections can also provide accurate prevalence and incidence that are important in the surveillance and monitoring of diseases. A parasitology laboratory should be able to:

- confirm a clinical impression that the condition has a parasitic nature;
- rule out differential diagnoses;
- aid a clinician in the choice of proper medication; and
- help in monitoring the effect of a treatment regimen.

The ability of a parasitology laboratory to generate reliable results is dependent on the proper collection, handling, and processing of specimens prior to examination, the skill of the laboratory analyst (examiner), and the quality of equipment used in the examination.

Diagnosis of parasitic infections is done either by the demonstration of parasite or parasite components (e.g., adults, eggs, larvae,

cysts, oocysts, trophozoites, and antigen) or by the detection of host immune response to the parasites (e.g., antibodies). It must be emphasized that demonstration of the parasite and/or the parasitic antigen provides a definitive diagnosis, while detection of the humoral immune response provides only presumptive evidence of infection.

Demonstration of parasites is possible only during the patent stage of the infection. There are cases where the parasite is not demonstrable even in active infection, as in schistosomiasis. In light infections and when parasites are still immature, recovery of parasites from infected individuals may not be possible. In such cases, immunoassays may become useful.

Among the specimens available for parasitic examinations, the stool is most commonly utilized. Other specimens like urine, blood, sputum, cerebrospinal fluid, tissue aspirate, tissue biopsies, and orifice swabs are also used for diagnosis. Fresh specimens in sufficient amounts are valuable in most instances.

The proper procedure can be determined if the diagnostician has a basic knowledge of the biology of the parasite. The life cycle will help in deciding which specimen to be collected, as well as the frequency and timing of collection. The parasite species and stage of development in the life cycle are also important factors to consider.

Examination of Stool or Fecal Sample

The most common method of diagnosis of intestinal parasites is through the demonstration of eggs, larvae, adults, trophozoites, cysts, or oocysts in the stool. Techniques are available where recovery of both helminthic and protozoan parasites is possible.

The fecal specimen is best collected in clean, wide-mouthed containers made of waxed cardboard or plastic with a tight-fitting lid to ensure retention of moisture and to prevent accidental spillage.

The stool specimen should be submitted with the following information:

1. patient's name
2. age
3. sex
4. date/time of collection
5. requesting physician
6. requested procedure
7. presumptive diagnosis
8. prior infections
9. travel history

For stool materials to be useful in parasitic diagnosis, there are important factors to consider:

- A. Intake of drugs/medicinal substances
1. antacids
 2. anti-diarrheals
 3. barium
 4. bismuth
 5. laxatives

All of these drugs have been found to leave crystalline residues that can interfere with the identification of parasites. Stool samples should be collected a week after the last intake of any of these drugs.

- B. Intake of antibiotics usually decreases the number of protozoans for several weeks.

- C. Amount of stool to be collected is dictated by the techniques that will be used. A routine stool examination usually requires a thumb-sized specimen of formed stool or about 5 to 6 tablespoons of watery stool.
- D. Contamination with toilet water, urine, or soil must be prevented since these can destroy protozoan trophozoites. In addition, soil and water may contain free-living organisms that would complicate diagnosis of infections.
- E. Age of the stool sample is very important for diarrheic specimens since the trophozoites it may contain are likely to die within 30 minutes to 1 hour after passage. Therefore, stools must be examined within that period of time.
- F. Delay in examination of specimens may require preservation to ensure that parasites are present in the identifiable stage.
- G. Temporary storage of fecal samples in a refrigerator (3-5°C) is acceptable, but prolonged refrigeration can bring about desiccation. Trophozoites are killed by refrigeration, although helminth eggs and protozoan cysts are usually not damaged.

NEVER FREEZE STOOL SAMPLES. NEVER KEEP THEM IN INCUBATORS.

Stool Preservatives

Appropriate fixation of parasites in the stool will preserve protozoan morphological features and prevent possible destruction of helminth eggs and larvae. Several stool preservatives are available, but only the more common ones will be discussed here. When selecting a fixative, the possibility of preparing a permanently stained slide should be considered. Stool samples must be adequately mixed with the selected

preservative in a proportion of one part stool to three parts preservative. Any of the following stool preservatives can be used:

1. **Formalin** is an all purpose fixative. A 5% concentration is recommended for protozoan cysts, while a 10% concentration is recommended for helminth eggs and larvae. The solution may be buffered with sodium phosphate to preserve the morphological characteristics of the organisms. Preserved stool can be concentrated using formalin-ether/ethyl acetate concentration technique (FECT).
2. **Schaudinn's solution** is used to preserve fresh stool in preparation for staining the stool smears. It contains mercuric chloride which is highly toxic to humans. Problems of mercury disposal may therefore arise.
3. **Polyvinyl alcohol (PVA)** is a plastic resin which serves to adhere a stool sample onto a slide. It is normally incorporated into the Schaudinn's solution, therefore the actual fixation is done by the Schaudinn's. The main advantage of using PVA is related to the preservation of protozoan cysts and trophozoites for permanent staining. Stools preserved in PVA can be concentrated using FECT and can be shipped to any laboratory for further examination. One major drawback of PVA is the use of mercuric chloride. Some laboratory technologists have suggested replacing this compound with cupric sulfate.
4. **Merthiolate-iodine-formalin (MIF)** contains merthiolate (also called thimerosal) and iodine which act as staining components, while formalin acts as the preservative. It is useful for the fixation of intestinal protozoans, helminth eggs, and larvae. Preserved

stools can be examined through the wet mount, but difficulty in the specific identification of protozoans may be encountered. The Lugol's iodine component should always be freshly prepared since it is unstable. Staining of preserved stools in MIF yields unsatisfactory results.

5. **Sodium acetate-acetic acid formalin (SAF)** has the advantage of not containing mercuric chloride. Images of organisms fixed in SAF, however, are not as sharp after staining compared with those fixed in PVA or Schaudinn's solution. It is a liquid fixative with a long shelf-life.

Methods of Examination

Stool samples are submitted to the laboratory in the fresh state or as preserved samples. If stools are fresh, the laboratory can classify the consistency of the stools as formed, semi-formed, soft, loose, or watery. The consistency can give an indication of the stage of the organism that may be present in the sample. Protozoan trophozoites are generally observed in soft or liquid stool, while the cysts are often found in formed or semi-formed samples.

On the other hand, helminth eggs and larvae can be found in any type of consistency. In watery samples, there may be a reduction in the number of eggs and larvae due to the dilution factor. Some authorities recommend the use of purged stools to increase the chances of recovering the protozoan trophozoites. Purged samples should be examined immediately after collection.

The color of the stool can be indicative of the presence of the parasite. Presence of blood should always be reported. Dark-colored blood suggests bleeding high up in the gastrointestinal tract, while bright red blood means bleeding from a more distal location. Blood and mucus in soft or watery stools may possibly yield the presence of trophozoites. Ingestion of some

compounds may impart a characteristic color to the stool (e.g., black color with iron intake).

By gross examination of the stools, tapeworm proglottids or adult nematodes like *Ascaris* or *Enterobius* may be found on or beneath the surface of the sample.

A. Microscopic Examination

Microscopic examination can reveal many elements present in the intestinal tract aside from parasites and normal fecal constituents. It is therefore highly recommended that a parasitology diagnostician be able to identify parasites apart from artifacts.

The following are elements that may be found in stool specimens in addition to parasites:

1. White blood cells:
 - a. Polymorphonuclears (PMNs), which may indicate inflammation
 - b. Eosinophils, which may indicate an immune response to a parasitic infection
2. Red blood cells, which may indicate ulcerations or bleeding
3. Macrophages are usually present in both bacterial and parasitic infections. In actual practice, one can mistake the active macrophages for amebic trophozoites.
4. Charcot-Leyden crystals are released with the disintegration of eosinophils. They may indicate presence of hypersensitivity or parasitic infections, especially amebiasis.
5. Epithelial cells from the intestinal tract can also be recovered.
6. Eggs of arthropods, plant nematodes, and other spurious parasites may be mistaken for human parasites.
7. Fungal spores coming from *Candida* spp., yeast, and yeast-like fungi may also be mistaken for parasites.

8. Elements of plant origin which resemble some parasites include:

- a. plant cells/fibers
- b. pollen grains
- c. starch granules
- d. vegetable spirals

9. Plant and animal hairs may look like helminth larvae.

Techniques

A. Direct Fecal Smear (DFS)

About 2 mg of stool (amount forming a low cone at the tip of an applicator stick) is comminuted thoroughly with a drop of 0.85% sodium chloride solution (NSS) and then covered with a cover slip.

This is a routine method of stool examination primarily useful in the detection of motile protozoan trophozoites. In this preparation, the trophozoites appear very pale and transparent. Trophozoites can be stained to demonstrate the nuclear morphology using Nair's buffered methylene blue (BMB) solution. *Entamoeba* cytoplasm will stain pale blue and the nucleus, darker blue.

Protozoan cysts can also be seen in a DFS. A weak iodine solution (Lugol's solution or D'Antoni) can be used as a temporary stain to demonstrate nuclei. Alternatively, a new mount can be prepared with iodine alone. The cytoplasm will stain golden yellow, the nucleus will be pale and refractile, and the glycogen will be deep brown. Helminth eggs and larvae can also be detected using this preparation. Because the amount of stool used in DFS is very small, light infections may not be detected.

Micrometry, as a tool to measure cysts and ova, will be useful in specific species identification.

B. Kato Thick Smear

About 50 to 60 mg of stool (approximately the size of two mung beans) is placed over a glass

slide and covered with cut cellophane paper soaked in a mixture of glycerine and malachite green solution. Glycerine is a clearing solution and malachite green is used to give color to the cellophane in order to give a pale green background to the eggs and to minimize the brightness of the microscopic field. If malachite green is not available, green cellophane soaked in glycerine may be used. The preparation is best examined within 10 to 20 minutes.

The technique is simple and economical, and is therefore useful in mass stool examinations. It is very good in detecting eggs with thick shells (e.g., *Ascaris* and *Trichuris*) but not eggs with thin shells (e.g., hookworm). In many instances, if the preparation is kept too long before examination, hookworm eggs become too transparent or distorted, making identification very difficult. Usefulness is limited if stools are diarrheic or watery. Likewise, it is not able to detect protozoan cysts and trophozoites.

C. Concentration Techniques

Concentration techniques can separate protozoan cysts and helminth eggs from a larger amount of stool (usually 1 g in amount) based on differences in specific gravity. In cases of light infections, or if there is a need to recover more parasites, stool concentration procedures are recommended. These procedures are based either on sedimentation or flotation. In sedimentation techniques, a parasite that has a higher specific gravity than the reagent will sink to the bottom of the preparation, while a parasite with a lower specific gravity will float to the surface. Mounts prepared from flotation techniques are cleaner than those from sedimentation.

1. Sedimentation Procedures

a. Acid Ether Concentration Technique (AECT)

The main reagents are 40% HCl, which can dissolve albuminous material, and ether, which can dissolve neutral fats in the stool. This technique is recommended for the recovery

of *Trichuris*, *Capillaria*, and trematode eggs, especially *Schistosoma*. This is also the choice if stool material comes from animals like cats and dogs. Drawbacks in the use of this technique include: loss of parasite to the plug of debris and possible destruction of protozoan cysts.

b. Formalin-Ether/Ethyl Acetate Concentration Technique (FECT)

This procedure makes use of 10% formalin which is an all purpose fixative, and ether, which can dissolve neutral fats in the stool. This is useful in the recovery of both helminth eggs and protozoan cysts. FECT can also be done with formalin-preserved and PVA-preserved stools. More parasites can be recovered from formalin-preserved samples. Parasite morphology is also better preserved in formalin than in PVA. Sediments from FECT can be stored for a long period of time.

The use of ether has been a cause for concern in the laboratory sector because of problems in storage and handling of this explosive and flammable compound. In place of ether, ethyl acetate may be used in sedimentation procedures. Those who have tried ethyl acetate claim that it is more efficient than ether in the recovery of cestode eggs and *Giardia* cysts. However, ethyl acetate is not as efficient as ether in the extraction of fat or mucoidal material from the stool.

2. Flotation Procedures

a. Zinc Sulfate (ZnSO_4) Flotation

The main reagent is a 33% zinc sulfate solution. Before use, the specific gravity should be checked. The ideal specific gravity ranges from 1.18 to 1.20. If parasites are exposed to high specific gravity, distortion and shrinkage of protozoan cysts and thin-walled nematode eggs may occur.

b. Brine Flotation

This makes use of a saturated table salt solution. Stools are directly mixed with the brine

solution. There is no need for centrifugation since helminth eggs rise to the surface of the solution. This technique is low-cost and simple but helminth eggs like hookworm and *Schistosoma* become badly shrunken. This is not useful for operculated eggs like *Clonorchis*, *Opisthorchis*, and heterophyids because these do not float in brine solution.

c. Sheather's Sugar Flotation

Boiled sugar solution preserved with phenol is used in this method. This technique is considered the best for the recovery of coccidian oocysts, mainly *Cryptosporidium*, *Cyclospora*, and *Cystoisospora*. With this procedure, visualization of oocysts can be better appreciated through the use of a phase microscope.

D. Stool Culture Methods

Ova of all hookworm species are similar, and speciation is therefore impossible to make species identification. Larval differentiation between hookworm and *Strongyloides* at the rhabditiform stage is possible but difficult. At the filariform stage, however, species identification can be done.

Stools positive for hookworm ova and/or *Strongyloides* rhabditiform larvae can be cultured until filariform larvae develop. This technique can also be used for *Trichostrongylus* sp.

1. Copro Culture

Positive stools are mixed with moistened soil or granulated charcoal. This simulates

environmental conditions in nature. Larvae are harvested using the Baermann procedure.

2. Harada-Mori or the Test Tube Culture Method

This technique makes use of test tubes and filter paper strips. Positive stool is applied to the filter paper and placed into a test tube with about 7 mL of boiled or distilled water. Filariform larvae will generally move downwards against the upward capillary movement of water and can therefore be recovered from the water at the bottom of the tube. On the other hand, *Strongyloides* larvae may instead move upwards and accumulate at the upper end of the filter paper strip.

Filariform larvae are infective and caution must be observed in handling stool cultures. Stools for culture should not be refrigerated because some species fail to develop when exposed to cold temperature.

Culture media are also available for the cultivation of the intestinal protozoan; however very few laboratories offer this service. Intestinal protozoans have been successfully cultivated in the laboratory but these culture methods are not recommended as substitutes for routine microscopic examination.

E. Egg Counting Procedures

Egg counting procedures may help correlate the severity of clinical disease with the intensity of infection or worm burden (Table 7.1). It is also done to assess the efficacy of anthelmintics

Table 7.1. WHO classification of intensity of infections with soil-transmitted helminths and *Schistosoma* spp.

Organism	Light intensity	Moderate intensity	Heavy intensity
<i>Ascaris lumbricoides</i>	1 – 4,999 epg	5,000 – 49,999 epg	≥ 50,000 epg
<i>Trichuris trichiura</i>	1 – 999 epg	1,000 – 9,999 epg	≥ 10,000 epg
Hookworm	1 – 1,999 epg	2,000 – 3,999 epg	≥ 4,000 epg
<i>Schistosoma japonicum</i> <i>Schistosoma mansoni</i>	1 – 99 epg	100 – 399 epg	≥ 400 epg

and the reduction of worm burden following treatment.

1. *Kato-Katz Method or the Cellophane Covered Thick Smear*

This procedure uses a measured amount of stool which has been sieved through a wire mesh and pressed under cellophane paper soaked in glycerine-malachite green solution. A uniform amount of stool is examined through the use of a template with a uniform-sized hole in the middle. All eggs seen in the whole preparation are counted. The total egg count is multiplied with a factor depending on the amount of stool used.

The procedure is useful for assessing the intensity of infection with *Schistosoma* and common soil-transmitted helminths like *Ascaris*, *Trichuris*, and hookworm.

Consistency of the stool is the main determinant for the sensitivity of this technique, since well-formed stools yield higher egg counts than moist ones. The technique can only be done on fresh formed stools and not on liquid and preserved samples.

For the identification of *Schistosoma* ova, 1% eosin solution can be layered over the cellophane paper. This method can help in the visualization of the miracidium.

2. *Stoll Egg Count*

This technique makes use of 0.1 N NaOH and a stool displacement flask calibrated at 56 mL and 60 mL. The sodium hydroxide acts as a stool diluent. It saponifies fat and frees eggs from fecal debris. The amount of diluted stool used for egg counting is measured by Stoll pipettes calibrated at 0.075 mL and 0.15 mL. The constant used to multiply the total egg count depends on the amount of stool examined.

Like the Kato-Katz method, sensitivity is determined by the consistency of the stool since formed stool can displace more sodium hydroxide than liquid stool. Aside from the

constant, there may be a need for a correction factor in computing for the egg count taking into consideration stool consistency.

F. Staining of Stool Specimen

Staining of stool specimen can also be done specifically in the examination of the nuclear characteristics of amebae. These are also useful in the identification of the other intestinal protozoans like *Balantidium* and *Giardia*. Techniques available include:

1. Iron-Hematoxylin
2. Trichome
3. Periodic Acid Schiff (PAS)
4. Chlorazol Black E

The abovementioned techniques are not very useful for the identification of coccidian oocysts like *Cryptosporidium*, *Cyclospora*, and *Cystoisospora*. For these parasites, Kinyoun's method of acid-fast staining is recommended.

Acid-fast staining of stool specimen requires spreading a thin layer of stool on a glass slide. The oocysts of the three coccidian parasites stain pink to red with a blue or green background. The background actually depends on the counter stain used. For *Cryptosporidium* and *Cyclospora*, oocysts are spherical, although *Cryptosporidium* has a diameter of 4 to 6 μm , while *Cyclospora* are 8 to 10 μm in diameter. On the other hand, *Cystoisospora* oocysts are more ovoid than spherical.

Generally, these organisms are recovered better from diarrheic and watery samples.

Perianal Swab

The perianal swab can be used to recover eggs of *Enterobius vermicularis* and *Taenia* spp. The *Enterobius* gravid female migrates out through the anus at night time, and deposits eggs on the perianal skin. *Taenia* spp. gravid segments can crawl out of the anus and in the process, ova are squeezed out of the segment and are deposited on the perianal skin.

A. Cellulose Tape or Scotch Tape Method

This is done by sampling the perianal skin using a strip of cellulose tape attached onto a glass slide. The sticky side is applied to the skin. The specimen can be collected early in the morning before the patient has taken a bath or before the patient has washed the perineum. Positive results have also been obtained from swabs collected late at night when patients have already slept for several hours.

Collected specimens are then examined under the microscope for the presence of eggs or the adult *Enterobius*. In some laboratories, a drop of toluene or xylene solution helps in the visualization of eggs.

Repeat examinations are recommended if results are negative.

Examination of Blood

Several species of helminthic parasites (e.g., filariae) and protozoan parasites (e.g., *Plasmodium*, trypanosomes, and *Babesia*) are in the blood at some stage of their life cycle. There are several techniques utilized for blood preparation and examination. Glass slides for blood examination must be absolutely clean and grease-free.

Methods

A. Finger-prick blood sample *must be free-flowing to prevent dilution of blood with tissue fluid, which decreases the number of parasites.*

1. Wet/fresh Preparation

Microfilariae and trypomastigotes are large and motile in fresh blood preparations. Their presence in the sample can therefore be easily detected. Species identification, however, is not possible with the wet mount.

2. Stained Smears

- Thick films* are prepared from two to three small drops of blood which are mixed and spread with continuous movement over

an area which is about 2 cm in diameter. Films are then thoroughly dried and then dehemoglobinized prior to staining.

- Thin smears* are prepared in such a way that they are thick at one end, and thin and feathery at the other end. Streaks and holes should be avoided in the film. Clean slides and spreaders are used. After air-drying, slides are fixed with methanol before staining.

Thick smears are used in the demonstration of microfilariae and rapid diagnosis of malarial infection. Thin smears are most useful in species identification of malarial parasites.

Stains that are usually used for blood parasites include: Giemsa stain, Wright's stain, and Delafield hematoxylin stain.

- **Giemsa stain** may be prepared from powder or may be commercially purchased as concentrated stock solution. With this stain, red cells stain pale red, white cell nuclei stain purple, eosinophils stain bright purple red, and neutrophils stain deep pink purple.
- **Wright's stain** already contains alcohol, so fixation is not needed before staining. Stained smears show light red erythrocytes, bright blue nuclei of leukocytes, bright red eosinophilic granules, and pink neutrophilic granules.
- **Delafield hematoxylin stain** is mainly useful in demonstrating the detailed structures of microfilariae. In this method, thick films are dehemoglobinized in 2% formalin with 1% acetic acid. The main stain is a mixture of hematoxylin and ammonium alum which enhances nuclear detail and morphological features. Another advantage of this method is that stained smears could be permanently mounted with Canada balsam or permount.

3. Capillary Tube Method

Finger-prick blood sample is collected using a heparinized capillary tube. The tube is sealed at one end and then centrifuged. After centrifugation, there will be three layers. At the bottom is the red cell layer, followed by the white cell layer called the buffy coat, and on top is the plasma. Microfilariae and trypanosomes can be readily visualized at the buffy coat area when the capillary tube is examined under a microscope.

a. Buffy Coat Films

The capillary tube can be broken at the area of the white cell layer after centrifugation of the capillary tube. The white cell layer can be spread and stained either with Giemsa or Wright's stain. Trypanosomes and *Leishmania* are concentrated at the buffy coat portion.

b. Quantitative Buffy Coat (QBC)

This method makes use of a capillary tube which is precoated with acridine orange and potassium oxalate. A cylindrical float is inserted to enlarge the layers. After centrifugation, the tube is read using an ultraviolet microscope. The DNA of the parasites takes up the acridine orange stain causing fluorescence among the non-fluorescing red blood cells. This method is useful in the demonstration of malaria parasites, microfilariae, trypanosomes, and *Babesia*.

B. Venous blood may be concentrated in order to detect microfilariae. Aseptic technique must be observed in the collection of the sample.

1. Knott's Concentration

In cases of low microfilaremia, 1 mL of blood can be mixed with 10 mL of 2% formalin and then centrifuged. The supernate is discarded and the sediment is studied. Part of the sediment can be spread like a thin blood film and stained.

2. Membrane Filtration

Like Knott's concentration, this method is also very useful when the density of microfilariae is low. This technique makes use of a syringe attached to a Swinney filter holder. One mL of fresh or anticoagulated blood is drawn up into the syringe and lyzed by adding 10 mL of distilled water. The lyzed blood is then passed through the Swinney membrane filter where microfilariae will be recovered. The membrane filter can be examined like a wet smear preparation or may be dried, fixed, and then stained.

Examination of Sputum

There are several parasites that may be recovered from the sputum. These include:

- A. Migrating larvae of *Ascaris lumbricoides*, *Strongyloides stercoralis*, and hookworms
- B. *Paragonimus* ova
- C. *Echinococcus granulosus* hooklets from pulmonary hydatid cysts
- D. Protozoa such as:
 1. *Entamoeba histolytica* trophozoites from pulmonary amebic abscess
 2. *Cryptosporidium parvum* oocysts, although very rare
 3. Non-pathogenic *Entamoeba gingivalis* and *Trichomonas tenax*

For most sputum examinations, the first morning specimen is considered the best specimen to examine. If the patient cannot expectorate, inductants like 10% sodium chloride or hydrogen peroxide may increase the amount of sputum collection. The specimen must be collected in disposable, impermeable, tightly covered containers and must be sent to the laboratory immediately.

Methods

A. Gross or Macroscopic Examination

1. **Consistency** of the sample as to serous, mucoid, purulent, bloody or combination, should be reported; and
2. **Color** may be indicative of cellular composition such as:

Yellow color may indicate pus; greenish tint may indicate *Pseudomonas* infection; while bright red color may indicate a recent bleeding rust color may indicate breakdown of hemoglobin.

B. Microscopic Examination

1. **Wet mount** using saline or iodine is useful when searching for protozoan trophozoites; and
2. **Sputum Concentration**

If the sputum is thick or viscous, an equal amount of 3% NaOH is added, thoroughly mixed, and then centrifuged. The supernate is discarded, and the sediment is studied as a wet mount.

Examination of Urine

Parasites have been reported from urine. Considered best for parasite recovery is urine collected first thing in the morning, since there could have been concentration of parasites overnight.

Urine is a very good specimen to study for the diagnosis of trichomoniasis vaginalis. The sample is centrifuged and the sediment is studied under the microscope. The organisms appear as rounded, globular, and transparent structures exhibiting jerky tumbling motion. Vaginal and urethral discharges are also used in the diagnosis of trichomoniasis.

Aside from *Trichomonas vaginalis*, some laboratories have reported recovery of *Wuchereria bancrofti* microfilariae from chyluric samples. With massive labor exportation to the Middle East, it is worth mentioning that the Filipino overseas contract workers may acquire *Schistosoma haematobium*. Eggs of this parasite are passed out with urine.

Examination of Tissue Aspirates

Samples aspirated from the following organs have been found to yield some parasites.

1. Liver
2. Duodenum
3. Bronchial
4. Lymph node
5. Skin

In the Philippines, the most common aspirate submitted for parasite diagnosis comes from the liver. It is usually requested to rule out hepatic amebic abscess. Demonstration of *Entamoeba histolytica* trophozoites is not easy especially in cases when the submitted material is aspirated from the center of the abscess where there is necrosis. The best material for this purpose is aspirate coming from the margin or the wall of the abscess.

In endemic countries, liver aspirate can be used in the recovery of hydatid sand, composed of intact and degenerating scolices of *Echinococcus granulosus*. While the parasite is generally believed to be absent in the Philippines, there are a few reported cases of infection in Filipino overseas contract workers assigned to endemic countries.

A. Duodenal Aspirate

There are occasions when duodenal aspirates are better specimens to use in the diagnosis of the following:

1. *Giardia lamblia*
2. *Strongyloides stercoralis*

Duodenal aspiration may be done through intestinal intubation but there is a simple and convenient procedure now available in the collection of duodenal contents. This is done through the "Entero Test," also known as the String test, where a capsulated yarn is swallowed by the patient. The yarn is expected to reach the duodenum. After about 4 hours, the yarn is retrieved and the mucoidal material clinging to the yarn is examined for the presence of the above mentioned parasites.

B. Cutaneous or Skin Aspirates

In very rare occasions, there may be requests to examine aspirates taken from cutaneous ulcerations, like in cases of cutaneous leishmaniasis. Like some of the parasites mentioned in other sections of this chapter, leishmaniasis is not supposedly endemic in the Philippines but due to exposure in endemic countries, there are reported leishmaniasis cases locally.

One clinical form of leishmaniasis is cutaneous, otherwise known as an Oriental sore. The recommended specimen is an aspirate taken from below the ulcer bed using a sterile needle. Smears are prepared and stained with Giemsa when dried. Positive samples will show the presence of amastigotes. In endemic countries, part of the needle aspirate can be inoculated into a culture medium.

Examination of Cerebrospinal Fluid (CSF)

Trypomastigotes of *Trypanosoma cruzi*, *Trypanosoma brucei rhodesiense*, and *Trypanosoma brucei gambiense* may be demonstrated in the CSF. Likewise, trophozoites of *Naegleria* may also be found in the CSF. In cases of parastromyiasis, CSF eosinophilia is a common finding, although there were reports that among infected children, *Parastromylus* larvae have been recovered.

Immediate examination of the CSF is required since trypomastigotes perish within

20 minutes, while the morphology and motility of *Naegleria* trophozoites are also affected within the same time period. The CSF must be centrifuged at 7,000 *g* for 10 minutes, the supernatant fluid discarded, and the parasites visualized from the sediment.

Examination of Tissue Biopsy Material

A. Muscle Biopsy

This specimen is very useful in the diagnosis of *Trichinella spiralis* infection, where small pieces of muscles are pressed between two glass slides and the preparation is examined under the microscope. Encapsulated larvae may be appreciated. While *Trichinella spiralis* is not present in the Philippines, larval infection with *Taenia solium* can result in cysticercosis, or a larval infection with *Spirometra* spp. can result in sparganosis. In both cases, muscle biopsy will be useful in the diagnosis of the conditions.

B. Rectal Biopsy

A more common biopsy material submitted for parasitic diagnosis is rectal biopsy. Examination of the rectal tissues can reveal the presence of deposited *Schistosoma japonicum* eggs.

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Examination of Tissues

Elia G. Paulino-Cabrera

Parasites may be an unexpected finding in tissues. A 60-year old male with headache thought to have a primary brain tumor was found to have cysticercosis (Plate 7.1). A 2-week old female with respiratory difficulty attributed to herpes turned out to have *Trichomonas vaginalis* of the nasopharynx.

In other cases, parasites may not be the cause of the symptoms but are seen together

with some other pathology. *Schistosoma* ova were incidentally found in the ovary and fallopian tube of a patient operated on for uterine leiomyoma (Plates 7.2 and 7.3). A hemicolectomy specimen with adenocarcinoma also had *Schistosoma* ova (Plate 7.4).

There are instances, however, when tissues are deliberately biopsied for the diagnosis of parasitic diseases. Because of the ease of



Plate 7.1. Cysticercus in brain
(Courtesy of Dr. Elia Paulino-Cabrera)

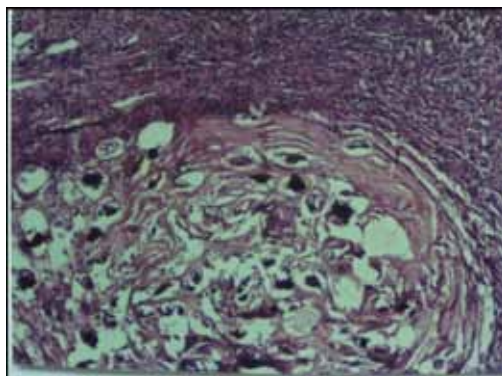


Plate 7.2. Ovary with incidental finding of
Schistosoma japonicum ova
(Courtesy of Dr. Elia Paulino-Cabrera)

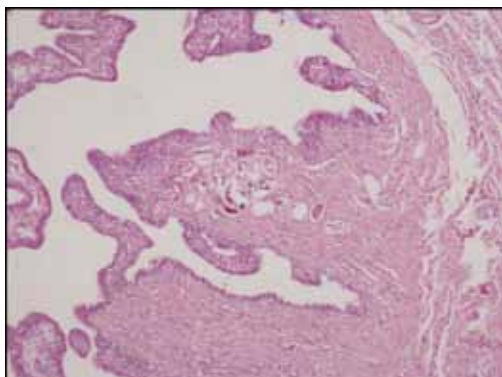


Plate 7.3. Fallopian tube with incidental finding of
Schistosoma japonicum ova
(Courtesy of Dr. Elia Paulino-Cabrera)

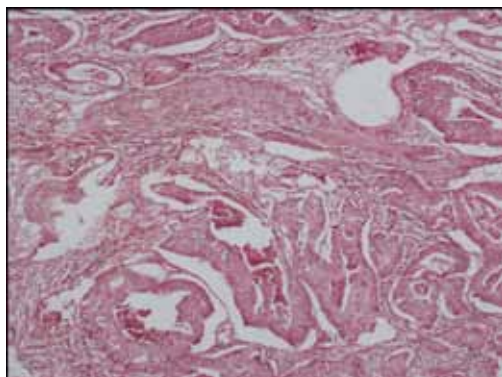


Plate 7.4. Colon with adenocarcinoma and
Schistosoma ova
(Courtesy of Dr. Elia Paulino-Cabrera)

obtaining other specimens like blood or stool for detection of parasites, tissues are usually not the initial specimens sent for diagnostic purposes. Biopsies are done when other specimens yield repeatedly negative results or when other tests are equivocal. For example, clinically suspected ameba cases with negative stool examinations may be definitely diagnosed by a direct smear or biopsy of the intestine. A biopsy may also be needed in the case of chronic schistosomiasis when the patient no longer excretes ova. Some parasites are found only in tissues, and biopsy is the best means of diagnosis. The presence of *Trichinella spiralis* larva in muscle, for instance, provides a definitive diagnosis of trichinellosis.

Virtually any organ of the body can be examined. Ova, larvae, adult forms, cysts, and trophozoites may all be seen. Before doing a biopsy, several factors should be considered. First is the nationality and travel history of the patient. Leishmaniasis, which is not endemic in the Philippines, should be a differential diagnosis in a patient with hepatosplenomegaly, lymphadenopathy, and a history of travel to the Middle East. The second factor is the life cycle and tissue trophism of the parasite. An adult nematode in a lymph node, for instance, is almost certainly a filarial worm (Plate 7.5). The accessibility of the biopsy site is a third

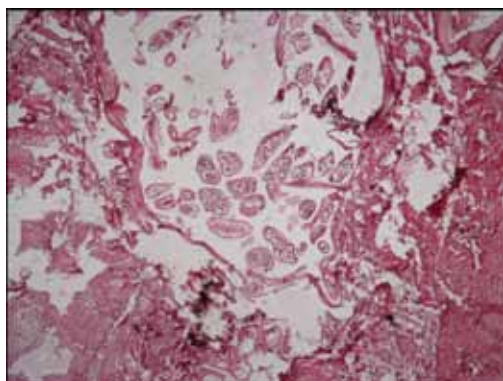


Plate 7.5. Adult filaria with microfilaria in an inguinal lymph node
(Courtesy of Dr. Elia Paulino-Cabrera)

factor. It is more practical to biopsy a skin mass than a visceral mass to document cysticercosis. A fourth factor is the possible complications of the procedure. A hepatic puncture is more likely to have complications than a lymph node biopsy in the diagnosis of visceral leishmaniasis.

Examples of commonly biopsied organs and parasites which may be found therein are shown in Table 7.2.

Table 7.2. Organs and parasites isolated

Organ	Parasite	
Skin and subcutaneous tissue	<i>Ancylostoma braziliense</i> (larva)	<i>Gongylonema</i>
	<i>Ancylostoma caninum</i> (larva)	<i>Loa loa</i> <i>Onchocerca</i> <i>Sparganum</i> <i>Strongyloides</i>
	<i>Cysticercus cellulosa</i> <i>Dracunculus</i> <i>Gnathostoma</i>	
Lymph node	<i>Filaria</i> <i>Leishmania</i>	<i>Trypanosoma</i>
Brain	<i>Cysticercus cellulose</i> Hydatid cyst	<i>Schistosoma</i>
Lung	Hydatid cyst	<i>Paragonimus</i>
Liver	<i>Entamoeba</i> Hydatid cyst	<i>Leishmania</i> <i>Schistosoma</i>
Small intestine	<i>Cryptosporidium</i>	Microsporidia
Rectum	<i>Balantidium</i>	<i>Schistosoma</i>
Muscle	<i>Cysticercus cellulosa</i> <i>Sarcocystis</i>	<i>Trichinella</i>
Eye	<i>Cysticercus cellulose</i> <i>Loa loa</i> <i>Onchocerca</i>	<i>Toxocara</i> <i>Toxoplasma</i>
Placenta	Malaria <i>Toxoplasma</i>	<i>Trypanosoma</i>
Bone marrow	<i>Leishmania</i>	<i>Trypanosoma</i> (amastigotes)

Definitive diagnosis depends on the identification of the parasites. The morphology of the ova, cysts, and trophozoites in tissues are similar to those in other specimens, such as stool. Diagnosis of metazoans in tissues is based on the demonstration of the following

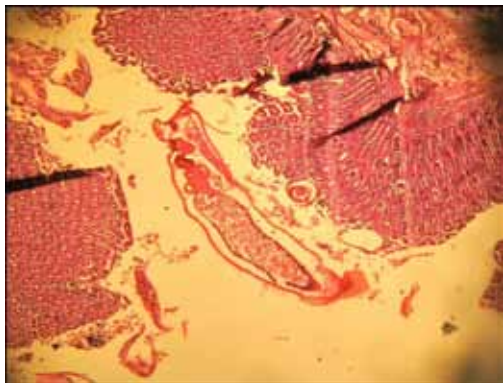


Plate 7.6. Adult *Trichuris* identified by ova in genital tract (Courtesy of Dr. Elia Paulino-Cabrera)

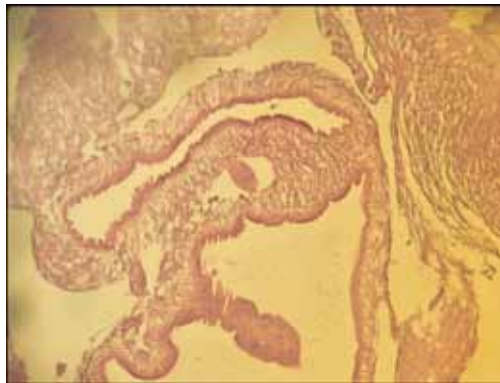


Plate 7.7. *Cysticercus* with calcareous corpuscles (Courtesy of Dr. Elia Paulino-Cabrera)

characteristics: (a) integument, (b) musculature, (c) body cavity, (d) digestive system, (e) reproductive organs and ova present (Plate 7.6), and (f) special glands or structures.

The integument may be chitinized (arthropods), striated (acanthocephala), spiny (platyhelminths), or smooth (nematodes). Muscles are described either as striated or smooth, and circular or longitudinal. Points of muscle attachment to the body and the number of cells per circumference are also noted. Meromyarian pertains to few cells (four or less), while polymyarian pertains to numerous cells per circumference. The body cavity is described according to content, which may be parenchymatous matrix, mesenchyme cells, or fluid. Of interest in the digestive tract are the pharynx and intestines. The number of branches of the pharynx and the number of intestinal cells should be noted. For the reproductive system, it should be determined whether the sexes are separate and whether the gonads are paired and tubular or sac-like. Special copulatory structures may be present. Examples of special structures which serve as diagnostic aids are the calcareous corpuscles (Plate 7.7) seen in cestodes, and reduplication of esophageal glands as seen in the group *Trichinellina*.

Tissue specimens may show not only the parasites themselves but also the body's reaction

to them. Grossly, organs may appear normal, enlarged, necrotic, or inflamed. Lesions may present as tumorous masses such as in an ameboma of the colon or echinococcosis of the liver or kidney. Fibrosis may cause hardening of the parenchyma, such as pipestem fibrosis in schistosomiasis. Microscopic findings may be varied as well. In some instances, no pathologic changes are evident. Intestines of patients with giardiasis and uncomplicated hookworm disease typically show normal-looking mucosa.

Acute reactions are present when there is tissue necrosis. These are exemplified by early amebiasis, ulcerated cutaneous leishmaniasis, trichomoniasis, and strongyloidiasis. Chronic inflammation is seen in any long-standing infection. A specific type of chronic infection is characterized by granuloma formation. Dead or degenerating parasites form the center of the lesion and are surrounded by lymphocytes, plasma cells, macrophages, multinucleated giant cells, and fibroblasts. Hyaline or eosinophilic material may be present. Several parasitic diseases show this reaction. Schistosomiasis and ascariasis lesions exhibit the characteristic Splendore-Hoeppli phenomenon. In filariasis, granulomas are known as Meyers-Kouvenaar bodies.

There are findings that are pathognomonic for some parasitic diseases. Lymph nodes in

toxoplasmosis are characterized by the presence of epithelioid histiocytes in the perifollicular zone. Malaria is characterized by the presence of hemozoin pigment in parasitized red blood cells. In the brain, the characteristic finding is Durck's granuloma, which consists of glial proliferations around capillaries.

Fine needle aspiration biopsy (FNAB) may be done prior to or in lieu of a formal biopsy. Parasite identification and host tissue response evaluation may be achieved with this method. Aspiration may be done on palpable lesions or under the guidance of ultrasound or computed tomography.

Biopsy specimens are placed in 10% buffered formalin for fixation. Many laboratories have automated tissue processors which allow slides to be completed the next day. Processing can also be done manually. Tissues are usually cut 3 μ m thick. Examination of serial sections may be needed before a diagnosis is made. Sometimes the parasite is simply too big that step sections are required to demonstrate different levels of organ systems.

Aspirates are smeared like peripheral blood smears. In aspirates which yield abundant fluid, a few smears should be made first. The rest of the specimen is preserved in an equal volume of 95% ethyl alcohol for cell block preparation. Touch imprints may be made on some specimens before fixation. Excess blood or fluid is blotted off from the surface before the specimen is pressed against a clean slide. Smears and imprints are fixed by placing slides in 95% alcohol for at least 15 to 20 minutes. Examples of parasites which may be demonstrated on touch imprints are *Toxoplasma* in placental tissues and *Leishmania* in lymph nodes.

Histopathologic slides are routinely stained with hematoxylin and eosin (H & E). Special stains may be needed to provide contrast between parasite and the background, or to highlight special structures. Periodic Acid Schiff (PAS) will demonstrate the cyst wall of *Toxoplasma* and the larva of *Toxocara*.

Trichrome stain has been found to be useful in differentiating the trophozoites of ameba and *Giardia* from host tissue. It also emphasizes structures such as flagella. Giemsa is used for different protozoa. Table 7.3 summarizes the parasites and special stains used for them.

Table 7.3. Special stains and corresponding parasites

Stain	Parasite	
Acid-fast	<i>Cryptosporidium</i> <i>Cyclospora</i>	<i>Isospora</i>
PAS	<i>Entamoeba</i> <i>Giardia</i> <i>Microsporidia</i> (polar granule)	<i>Toxocara</i> <i>Toxoplasma</i> (bradyzoites) <i>Trichomonas</i>
Giemsa	<i>Giardia</i> <i>Malaria</i>	<i>Toxoplasma</i>
GMS	<i>Microsporidia</i>	<i>Toxoplasma</i> (cyst wall)
Gram stain	<i>Microsporidia</i>	<i>Trichomonas</i>
Luxol Fast Blue	<i>Microsporidia</i>	
Reticulum	<i>Leishmania</i>	
Trichrome	<i>Balantidium</i> <i>Dirofilaria</i>	<i>Entamoeba</i> <i>Giardia</i>

Immunohistochemistry can be used as an adjunct in difficult cases. Immunologic techniques and principles are applied. Parasite protein is identified using anti-parasite monoclonal or polyclonal antibody.

An ordinary light microscope is used to examine tissue slides. Sometimes, polarizing, phase contrast, immunofluorescent and electron microscopy are used as well. Ova of *Paragonimus*, spores of *Microsporidia*, and hooklets of *Echinococcus* are birefringent and are demonstrable under polarizing light. Polarization microscopy also increases detection of hemozoin pigment in placentas.

Trichomonads and microsporidians are enhanced by phase microscopy. Immunofluorescent microscopy is used in *Trichomonas* infection. With acridine orange as the stain, fluorescent microscopy is more reliable

than wet mount or culture. It has also been used as an adjunct in the diagnosis of Chagas disease. Ultrastructure of human parasites, especially of protozoa, has been described. Electron microscopy is employed for the definitive diagnosis and speciation of microsporidiosis. The first case of cyclosporiasis was demonstrated by light microscopy. However, sporozoite, trophozoite, schizont, and merozoite stages were identified by electron microscopy. Specimens for electron microscopic studies are fixed in 3% glutaraldehyde in phosphate buffer.

A tissue sample with parasites may also be sent for frozen section diagnosis, usually with an initial or a working impression of malignancy. Preliminary diagnosis of the presence of a parasite is usually satisfactory for surgeons. Definitive diagnosis can be made after routine processing.

Tissues from autopsy cases are treated in the same way as biopsy specimens. Representative sections from organs are taken, fixed, processed, and stained as in biopsy specimens. Whole organs may be kept in jars for future references or as museum materials.

There are limitations to tissue diagnosis of parasites. Parasites may degenerate, fibrose or calcify. Severe inflammation or necrosis may mask them. Artifacts may render identification difficult. For instance, formalin pigment may be difficult to differentiate from hemozoin. Tissues may also be contaminated by bacteria or fungi which may show up in the slides. In such cases, other diagnostic modalities like immunoassays, molecular-based techniques and proteonomics using mass spectrometry may be useful.

Criteria for the histopathologic diagnosis of parasitic diseases like toxoplasmosis have been proposed in the absence of parasites. These need further evaluation and correlation with serologic findings.

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Advances in Diagnostic Parasitology

Maria Cielo J. Pasay

Diagnosis of parasitic diseases relies on laboratory diagnosis to complement clinical symptoms, clinical history, and travel history of the patient. While microscopic demonstration of parasites remains the only tool available in resource poor settings, recent developments in the diagnosis of parasitic infections provide promising alternatives. This chapter will highlight new developments in diagnostic parasitology.

Microscopy

Microscopy as a tool to diagnose parasitic diseases remains as the gold standard in most laboratories especially in the diagnosis of common helminth and protozoan infections. It is simple as it allows direct detection of parasites, and informative as morphologically distinct parasites are readily differentiated. However, to achieve a good level of sensitivity, microscopy requires high parasite density in the clinical specimen being examined. Parasites can be low in numbers during pre-patent and chronic periods of infection, hence, microscopic examination may yield false negative results. Parasite concentration techniques such as the FLOTAC method can be used prior to microscopic examination, but these require additional equipment, supplies, and reagents. *In vitro* culture methods may enhance recovery of parasites; however, a biosafety cabinet and special culture media are required, and results are not readily available. The use of an ultraviolet (UV) fluorescent microscope can also improve detection of parasites in wet mount preparations. With fluorescence microscopy, *Cyclospora* oocysts exhibit intense blue color in contrast to refractile spheres with distinct oocyst wall in bright field microscopy. While the application of fluorescent techniques increases

the sensitivity of microscopic diagnosis of parasitic diseases, an experienced microscopist is required. The sensitivity of microscopic diagnosis is highly dependent on the level of training and experience of a microscopist for accurate identification of the parasitic agent. Specimen preparation for microscopic examination can also be laborious and tedious when a lot of samples need to be examined during epidemiologic investigations. Therefore, in parasite endemic regions with limited resources, misdiagnosis using the microscope may compromise patient care.

Immunodiagnosis

To overcome problems related to microscopic examination of parasites, immunodiagnostic techniques provide useful alternatives. A number of immunodiagnostic tests for parasitic infections are available that detect either antigen or antibodies in clinical specimens. These include immunofluorescent assay (IFA), enzyme-linked immunosorbent assay (ELISA), hemagglutination test (HA), and immunoblotting (dot blot). These methods are also useful in monitoring response to chemotherapy.

A. Detection of Antibodies

Tests to detect antibodies against the parasite in question are used when biologic specimens do not permit microscopic diagnosis during chronic or asymptomatic infections. They are also recommended in parasitic infections where direct identification of parasites in host deep tissues is not generally possible such as in toxoplasmosis or toxocarasis. Detection of antibodies is also a useful alternative in the diagnosis of cysticercosis or echinococcosis where invasive techniques to obtain specimen

for diagnosis can pose some risk to the patient. A positive antibody test can be a useful indicator of a recent infection if the patient has no previous exposure to the parasite prior to travel in an endemic area. In contrast, positive antibodies in a resident of an endemic area may reflect either past or current infection with a specific parasite under consideration. Therefore, parasite diagnosis based on positive antibodies can only be indicative of infection at some indeterminate time and not necessarily current or acute infection. In addition, antibody tests are useful when significant levels of antibodies are produced with parasitic infections. In some people, parasitic infections may not stimulate antibody response or seroconversion may be delayed with onset of clinical symptoms.

Antibody detection assays use whole parasites from animal models or in vitro cultures or soluble crude extracts as antigens. Better sensitivity is achieved with the use of these

antigens as they provide a larger repertoire of antigens recognized by the immune system. However, different types of antigen preparation (such as native protein, purified peptides, and recombinant proteins) may also produce variable antibody results. The use of a mixture of antigens can increase antibody detection but cross reactivity between parasite species cannot be ruled out leading to false positive results.

Given these limitations, the results of antibody tests in the diagnosis of parasitic infections must be interpreted with caution. The greatest utility of antibody tests is in investigating etiology of disease outbreaks and in epidemiologic investigations to map foci of disease transmission essential to institute control measures.

There are a number of antibody tests available for the diagnosis of parasitic diseases at the Centers for Disease Control and Prevention (CDC), USA (Table 7.4). There are only a few

Table 7.4. Antibody detection tests offered at CDC

Disease	Organism	Test
Amebiasis	<i>Entamoeba histolytica</i>	Enzyme immunoassay (EIA)
Babesiosis	<i>Babesia microti</i> <i>Babesia</i> spp. WA1	Immunofluorescence (IFA)
Chagas disease	<i>Trypanosoma cruzi</i>	IFA
Cysticercosis	Larval <i>Taenia solium</i>	Immunoblot (Blot)
Echinococcosis	<i>Echinococcus granulosus</i>	EIA, Blot
Leishmaniasis	<i>Leishmania braziliensis</i> <i>L. donovani</i> <i>L. tropica</i>	IFA
Malaria	<i>Plasmodium falciparum</i> <i>P. malariae</i> <i>P. ovale</i> <i>P. vivax</i>	IFA
Paragonimiasis	<i>Paragonimus westermani</i>	Blot
Schistosomiasis	<i>Schistosoma</i> spp. <i>S. japonicum</i> <i>S. haematobium</i> <i>S. mansoni</i>	FAST-ELISA Immuno Blot
Strongyloidiasis	<i>Strongyloides stercoralis</i>	EIA
Toxocariasis	<i>Toxocara canis</i>	EIA
Toxoplasmosis	<i>Toxoplasma gondii</i>	IFA-IgG, EIA-IgM
Trichinellosis (Trichinosis)	<i>Trichinella spiralis</i>	EIA

(Source: Division of Parasitic Diseases, Centers for Disease Control, Atlanta, Georgia, USA)

commercially available antibody detection tests to diagnose blood-borne parasitic infections such as malaria and filariasis, intestinal parasitic diseases such as giardiasis, cryptosporidiosis, schistosomiasis, and cysticercosis.

B. Detection of Antigen

A more sensitive and specific immunodiagnostic test to determine the disease status of patients is the detection of specific parasite antigens. Antigen detection in serum or whole blood (for blood parasites) and in feces, urine, duodenal fluid or biopsy specimens from the small intestine or urine (for intestinal parasites) is commonly achieved by immunocapture utilizing two antibodies. The first antibody (either monoclonal or polyclonal) is immobilized in a solid phase such as a microtiter plate or nitrocellulose membrane. This will capture the parasite antigen which is detected by the second antibody, usually a monoclonal antibody labeled with an enzyme. A colored reaction is observed after the addition of an enzyme substrate. Antigen detection tests have quicker turnaround times than microscopy and do not require experienced microscopists. To date, much research work has been achieved towards development and optimization of parasite antigen tests that resulted in commercially available reagents/kits for intestinal parasites such as *Cryptosporidium* spp., *E. histolytica*, *Giardia intestinalis*, and *Trichomonas vaginalis* (Table 7.5).

Several commercially available kits for the detection of *Cryptosporidium* antigens come in different formats such as enzyme immunoassay (EIA), direct immunofluorescence (DFA), or IFA. These kits detect either *Cryptosporidium* alone, or combinations involving *Cryptosporidium* and *Giardia* or *Cryptosporidium*, *Giardia*, and *E. histolytica*. The choice of test will depend on particular need for single tests in clinical settings or batch testing in epidemiological investigations or research. Sensitivities and specificities of commercially

available kits range from 93 to 100% when used in clinical settings. Some EIA tests come in microplate format and are robust enough to detect *Cryptosporidium* antigens either from fresh, frozen or preserved stool samples in either formalin or sodium acetate-acetic acid-formalin (SAF). However, concentrated or polyvinyl alcohol (PVA)-treated samples are not suitable for EIA testing. Combined antigen detection of either *Cryptosporidium* and *Giardia* or *Cryptosporidium*, *Giardia* and *E. histolytica* are also commercially available as rapid immunochromatographic assays in fresh or preserved stool specimens. As the name implies, rapid tests have the advantage of quickest turnaround time and the least requirement for an experienced laboratory personnel. They also offer the convenience of multiple results in one reaction device without the need for special equipment. Both EIA and rapid test kits show good correlation with DFA, which is reported to be the most sensitive and specific test in the diagnosis of cryptosporidiosis. It uses a fluorescein isothiocyanate (FITC)-labeled monoclonal antibody which detects antigens on the surface of *Cryptosporidium* oocysts in either concentrated or unconcentrated fecal samples.

Pathogenic *E. histolytica* and commensal *E. dispar* are morphologically identical. Antigen detection tests that differentiate the two species eliminate unnecessary treatment of patients. Commercially available diagnostic kits are mostly enzyme-based assays using monoclonal antibodies that detect galactose adhesins of the pathogenic *E. histolytica*. The Techlab *E. histolytica* II specific for *E. histolytica* was found to be highly sensitive and specific by several studies conducted in at least five countries in the world. A major drawback in using this kit in the diagnosis of intestinal amebiasis is the requirement for fresh, unpreserved fecal sample. Extraintestinal manifestations of amebiasis such as amebic liver abscess (ALA) on the other hand can be diagnosed by serology. Detection of Gal/GalNac lectin antigen in serum provides early

Table 7.5. Commercially available parasite antigen detection tests

Organism	Kit name	Manufacturer-distributor	Type of test
<i>Cryptosporidium</i> spp.	Crypto CELISA	Cellabs	EIA
	PARA-TECT™ Cryptosporidium Antigen 96	Medical Chemical Corporation	EIA
	ProSpect Rapid	Remel	EIA
	ProSpect	Remel	EIA
	Cryptosporidium	Techlab	EIA
	Cryptosporidium	Wampole	EIA
	Crypto CEL	Cellabs	IFA
	XPect Crypto	Remel	Rapid
<i>Cryptosporidium</i> spp./ <i>Giardia lamblia</i>	PARA-TECT™ Cryptosporidium/ Giardia DFA 75	Medical Chemical Corporation	DFA
	Merifluor	Meridian	DFA
	ProSpectT	Remel	EIA
	Crypto/ GiardiaCEL	Cellabs	IFA
	ColorPAC*	Becton Dickinson	Rapid
	ImmunoCard STAT!*	Meridian	Rapid
	XPect	Remel	Rapid
<i>Cryptosporidium</i> spp./ <i>Giardia lamblia</i> / <i>Entamoeba histolytica</i> / <i>Entamoeba dispar</i>	Triage	BioSite	Rapid
<i>Entamoeba histolytica</i>	Entamoeba CELISA	Cellabs	EIA
	E. histolytica	Wampole	EIA
	E. histolytica II	Techlab	EIA
<i>Entamoeba histolytica</i> / <i>Entamoeba dispar</i>	ProSpect	Remel	EIA
<i>Giardia duodenalis</i>	Giardia CELISA	Cellabs	EIA
	PARA-TECT™ Giardia Antigen 96	Medical Chemical Corporation	EIA
	Giardia II	Techlab	EIA
	Giardia	Wampole	EIA
	GiardiaEIA	Antibodies, Inc	EIA
	Giardia CEL	Cellabs	IFA
	ProSpectT	Remel	Rapid
	Simple-Read Giardia	Medical Chemical Corporation	Rapid
<i>Trichomonas vaginalis</i>			DFA
			EIA
			Latex Agglutination
<i>Wuchereria bancrofti</i>	Filariasis CELISA	Cellabs	EIA
	ICT Filariasis	Binax	Rapid

(Source: Division of Parasitic Diseases, Centers for Disease Control, Atlanta, Georgia, USA)

diagnosis of ALA and can be used as a test of treatment efficacy. Additionally, the presence of lectin in saliva can also be used as a predictor for invasive disease with the advantage of noninvasive sample collection.

Commercially available immunodiagnostic tests for diagnosis of giardiasis are in the same format as the diagnostic test kits for the diagnosis of cryptosporidiosis and amebiasis. The same requirement for unpreserved stool specimen applies for enzyme-based assays for the diagnosis of giardiasis. Detection of *Giardia* cysts by DFA assay employs FITC-labeled monoclonal antibody which is highly sensitive and specific compared to microscopy.

Molecular Diagnosis

Nucleic acid-based assays offer greater sensitivity and specificity than the above mentioned tests. They allow for direct detection of parasites in samples including those with very low parasite load from asymptomatic patients. The use of gene amplification technology by polymerase chain reaction (PCR) detects nucleic acid sequences specific to the parasite in question. This technique uses two oligonucleotide primers which flank the parasite target sequence and *Taq* polymerase. The process involves successive cycles of DNA denaturation, annealing of primers, and extension to generate an exponential number of copies of the target sequence using a thermocycler. The amplified target is then analyzed by gel electrophoresis or alternatively, by ELISA methods. Several variations of the traditional PCR have been developed to increase sensitivity such as nested PCR where a second round of amplification is introduced using a set of primers internal to the target sequence; multiplex PCR using parasite/species-specific primer sets to detect/differentiate parasite/species simultaneously in one reaction tube; and real-time PCR to quantify original template concentration by using various fluorescence chemistries such as SYBR Green, sequence-specific TaqMan probes,

fluorescence resonance energy transfer (FRET), and Scorpion primers.

The principle of real-time PCR using two fluorescence chemistries is illustrated below:

The principle of SYBR Green detection in real-time PCR is outlined in Figure 7.1. The fluorescent dye SYBR Green is added to the PCR mixture (1). SYBR Green is a DNA binding dye that fluoresces strongly when bound to double-stranded DNA. At the start of the reaction, very little double-stranded DNA is present, and so the fluorescent signal detected by the thermocycler is low (3). As the reaction proceeds and PCR product accumulate, the amount of double-stranded DNA increases and with it the fluorescence signal (4-5). The signal is only detectable during annealing and extension, since the denaturation step contains predominantly single-stranded DNA (6).

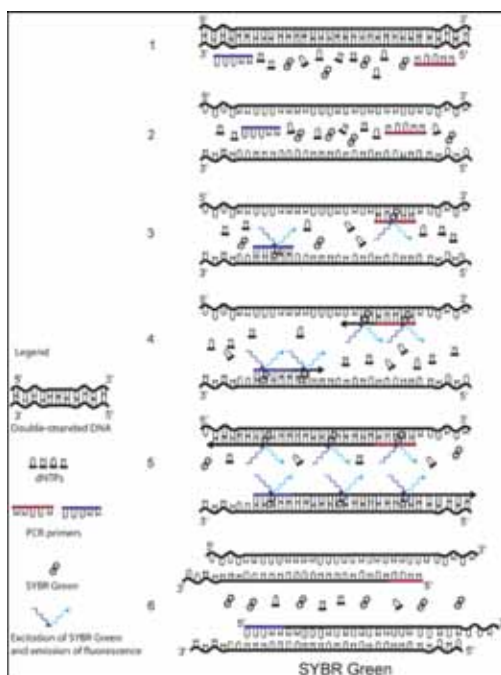


Figure 7.1. SYBR Green detection in real-time PCR (From da Silva A, Pieniazek N. Latest advances and trends in PCR-based diagnostic methods. In: Dionisio D, editor. Textbook-Atlas of Intestinal Infections in AIDS. Springer; 2003. p. 397-412.)

The principle of TaqMan real-time PCR is depicted in Figure 7.2. The TaqMan probe is designed to be complementary to a specific sequence spanned by the PCR primers. The TaqMan probe has a reporter dye at its 5' end and a quencher dye at its 3' end. As long as the probe is intact and the reporter and the quencher dyes are in close proximity, no fluorescence signal is emitted due to the quenching effect (black arrow in 1, 2, and 3) (1). After the annealing of the TaqMan probe (2) and the primers (3), the primers are extended by the DNA polymerase. As the polymerase reaches the TaqMan probe, it uses its exonuclease activity to remove the probe one nucleotide at the time (4). This releases the reporter from the proximity of the quencher and allows for the release of a fluorescence signal from the reporter (5).

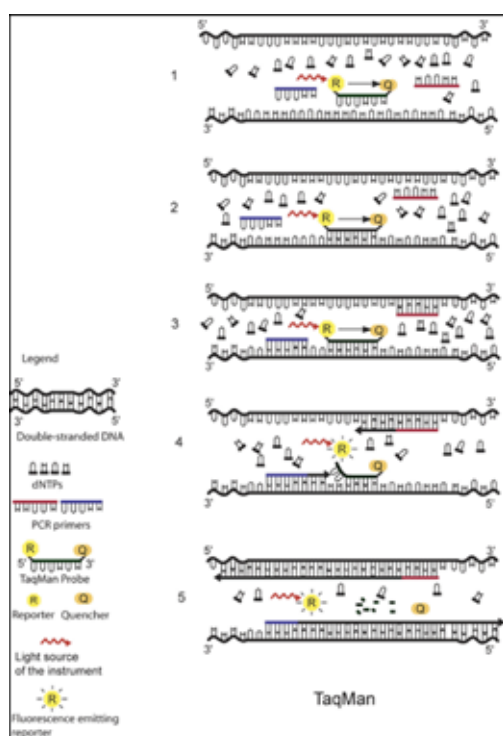


Figure 7.2. TaqMan real-time PCR
(From da Silva A, Pieniazek N. Latest advances and trends in PCR-based diagnostic methods. In: Dionisio D, editor. Textbook-Atlas of Intestinal Infections in AIDS. Springer; 2003. p. 397-412.)

Real-time PCR assays using SYBR Green are simpler and less expensive than TaqMan probe assays. However, all fluorescence bound to double-stranded DNA are detected, including primer-dimers and other PCR artifacts. Caution should be exercised when analyzing data resulting from this assay. To improve specificity, a melt/dissociation curve analysis should be included to distinguish real PCR products from artifacts. Probe-based assays on the other hand, are highly specific and can detect multiple targets in one tube.

Other new molecular approaches in the diagnosis of parasitic diseases such as loop-mediated isothermal amplification (LAMP) and Luminex-based technologies are also currently available. LAMP reactions are easier to set up as they do not require extraction of parasite DNA. The specimen of interest is mixed with diagnostic primers, substrates, and DNA polymerase capable of strand displacement in a microcentrifuge tube. Large quantities of pyrophosphate ions are produced during the reaction forming white precipitates. The resulting turbidity is proportional to the amount of DNA synthesized which can be measured in real-time or by the naked eye. Unlike a conventional PCR, LAMP is carried out at a constant temperature (usually 60-65°C) therefore eliminating the need for a thermocycler. LAMP can also be multiplexed for simultaneous detection and differentiation of parasite species. Because of its simplicity, the use of LAMP technology in the diagnosis of parasitic diseases in peripheral laboratories shows promise.

The Luminex xMAP Technology is another new method that allows for high throughput diagnosis of parasitic diseases in large scale studies, but is applicable only in central laboratories. It is a bead-based flow cytometry assay that allows for simultaneous detection of different targets (parasite species or genotypes) in the same reaction using very low volumes. The microsphere beads are covalently bound to antigens, antibodies or oligonucleotides and

used as probes in the assay. This assay is very useful in parasite genetic diversity and drug resistant allele studies.

Molecular Diagnosis of Stool Specimens

At the CDC, both conventional and real-time PCR analysis are currently used to detect *Cryptosporidium* spp., *Cyclospora cayetanensis*, *E. histolytica*, and *E. dispar*, while conventional PCR is used to detect *Giardia duodenalis* and microsporidia. DNA is extracted from fecal samples and diagnostic primers are used to amplify target gene or sequence. Amplification products of conventional PCR are loaded in agarose gels and analysed. Real-time PCR, on the other hand, measures the fluorescence signal in the reaction tube per cycle and is proportional to the amount of accumulated amplified product. The concentration of amplified DNA is measured by comparing it to a standard curve.

A TaqMan-based real-time PCR has been developed and validated at the CDC which differentiates *Cryptosporidium hominis* from *Cryptosporidium parvum*. The assay combines a generic TaqMan assay which targets the 18S rRNA to detect *Cryptosporidium* species and two other TaqMan assays to identify *C. hominis* and *C. parvum*. The generic TaqMan assay can detect one to 10 oocysts in a 300 µL stool specimen, and the two species-specific TaqMan assays are ten-fold more sensitive. These are valuable tools in outbreak investigations of cryptosporidiosis.

A single-tube multiprobe real-time PCR assay can simultaneously detect the pathogenic *E. histolytica* and the non-pathogenic *E. dispar*. The assay uses two species-specific probes encompassing new SSU RNA regions of the ribosomal DNA-containing episome. It is a highly sensitive assay capable of detecting one *Entamoeba* per mL of feces and is therefore more sensitive than a conventional nested PCR method. A multiplex real-time PCR assay can simultaneously detect *E. histolytica*, *Giardia intestinalis* and *Cryptosporidium* spp. in one tube using parasite-specific probes. The

primers and TaqMan probes for *E. histolytica* and *Giardia intestinalis* were designed on a small subunit ribosomal RNA gene, while those of *Cryptosporidium* spp. were designed on *Cryptosporidium* oocyst wall protein (COWP). This assay was found sensitive and specific when validated with clinical specimens.

Another multiplex real-time PCR assay using primers and probes targeting the cytochrome C oxidase gene of *Schistosoma* can detect and quantify two important species (*S. mansoni* and *S. haematobium*) in fecal samples. Real-time PCR cycle threshold (CT) values representing parasite/species DNA extracted from fecal material show good correlation with egg counts of *S. mansoni* in stool and egg counts of *S. haematobium* in urine.

Recently, a rapid diagnostic multiplex PCR (RD-PCR) to distinguish *S. haematobium*, causing human schistosomiasis from *S. bovis*, causing schistosomiasis in cattle was developed. There is a sympatric occurrence of these two species in Africa and they have the ability to infect the same intermediate snail host, *Bulinus*, thus, there is a need for a reliable method to differentiate the larval stages of the parasite. This assay uses a single forward primer and two species-specific reverse primers targeting the cytochrome oxidase subunit 1 (COX 1) mitochondrial DNA (mtDNA) which gives a 306 bp PCR product for *S. bovis* and 543 bp PCR product for *S. haematobium*.

Several molecular methods of detection and differentiation of *Taenia* species in stool samples have been developed. These include PCR restriction fragment length polymorphism (PCR-RFLP), multiplex PCR targeting mitochondrial DNA, and nested PCR method targeting Tso31 gene encoding the *T. solium* oncosphere-specific protein. A simple but highly sensitive and specific LAMP technology, on the other hand, targets COX 1 and cathepsin L-like cysteine peptidase (*clp*) genes for differential detection of *Taenia* species. This method utilizes a *Bst* DNA polymerase with strand replacement activity and four primers that recognize six

sequences on the target DNA under isothermal conditions. DNA prepared from proglottids, cysticerci, and fecal samples of taeniasis patients can be used for this assay.

Molecular Diagnosis of Blood Specimens

A highly sensitive multiplex real-time PCR assay has been shown to detect the five human *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*) in a single reaction tube even in samples with very low parasitemia. This method has been optimized for the detection of mixed infections with the increased sensitivity of detecting minor species by using species-specific forward primers in combination with a conserved reverse primer. It also provides great advantage over standard microscopy as it allows quick turnaround time and reduces cost per assay in large scale investigations. Multiplex real-time PCR can also be used in differentiating drug-sensitive from drug-resistant strains of *Plasmodium*, important in instituting malaria treatment.

LAMP technology was recently used in the diagnosis of malaria by targeting the 18S rRNA gene to simultaneously detect the four human *Plasmodium* species. When compared to nested PCR in the diagnosis of malaria, LAMP demonstrated a similar level of sensitivity, greater specificity, and a faster turnaround time.

Three LAMP assays based on *SAG1*, *SAG2*, and *B1* genes of *Toxoplasma gondii* are highly specific and sensitive, and allow rapid detection of active toxoplasmosis compared to conventional nested PCR. The lowest limit of detection of these LAMP assays is 0.1 tachyzoite, and they do not cross react with DNA of other parasites.

Malaria and lymphatic filariasis are co-endemic in many tropical and sub-tropical regions such as Southeast Asia, Western Pacific, Africa, South and Central America. As such, other diagnostic tests have been developed to complement microscopic examination of stained blood smears to detect *Plasmodium* spp. and *Wuchereria bancrofti*. Circulating

filarial antigens are detected by either ELISA or immunochromatographic test (ICT). Several PCR-based assays are available to diagnose malaria or Bancroftian filariasis separately. In areas where the two parasitic diseases are co-endemic, a multiplex PCR assay can be used to simultaneously detect *P. falciparum* and *W. bancrofti* in humans and a real-time multiplex quantitative PCR assay to detect *P. falciparum* and *W. bancrofti* or *P. vivax* and *W. bancrofti* in mosquitoes. Recently, a multiplex, post-PCR oligonucleotide ligation detection reaction-fluorescent microsphere assay (LDR-FMA) was developed for simultaneous detection of four *Plasmodium* spp. and *W. bancrofti* in blood samples. This methodology is very useful in the conduct of large scale epidemiologic investigations in areas where malaria and Bancroftian filariasis are co-endemic.

PCR-based assays are capable of detecting very low parasite loads, making them more sensitive methods of diagnosis. Their quick turnaround times offer the benefit of early diagnosis and treatment of patients. Efficacy of treatment can be monitored as a decrease in parasite DNA concentrations by quantitative real-time PCR; however, results should be interpreted with caution as they may not necessarily mean non-viability of the parasite in question. The chances of false negatives due to presence of PCR inhibitors that may be present in blood and other clinical specimens and false positives due to carry-over contamination should not be overlooked. In this regard, proper standardization procedures are needed for more reliable and reproducible results. Without these, PCR-based assays cannot be routinely used and may be limited to in-house research use only.

Rapid Diagnostic Tests (RDTs)

While molecular-based assays show excellent sensitivity, specificity, and rapidity than other methods of diagnosis of parasitic diseases, their use is still uncommon in daily laboratory practice especially in rural endemic areas where cases of parasitic infections are

concentrated. Early diagnosis and treatment of any parasitic disease are essential components of control programs, hence the continued development of diagnostic tests that can be performed on site without the need for electricity, sophisticated equipment, or extensive training of laboratory personnel. The use of Rapid Diagnostic Tests (RDTs) therefore has great potential in improving diagnostic accuracy of parasitic infections in field settings that still rely on the microscope.

RDTs use antibodies (monoclonal or polyclonal) to detect parasite antigens in blood,

stool, urine or other body fluids. These assays employ immunochromatographic methods in lateral flow devices where results are available within 15 minutes. They do not require skilled microscopists but provide accurate diagnosis in a timely manner important for prompt and appropriate treatment.

A. RDTs for malaria

A malaria RDT (Figure 7.3) is a lateral flow immunochromatographic device that detects protein [antigen (Ag)] derived from the blood stage of malaria parasites. Blood is usually

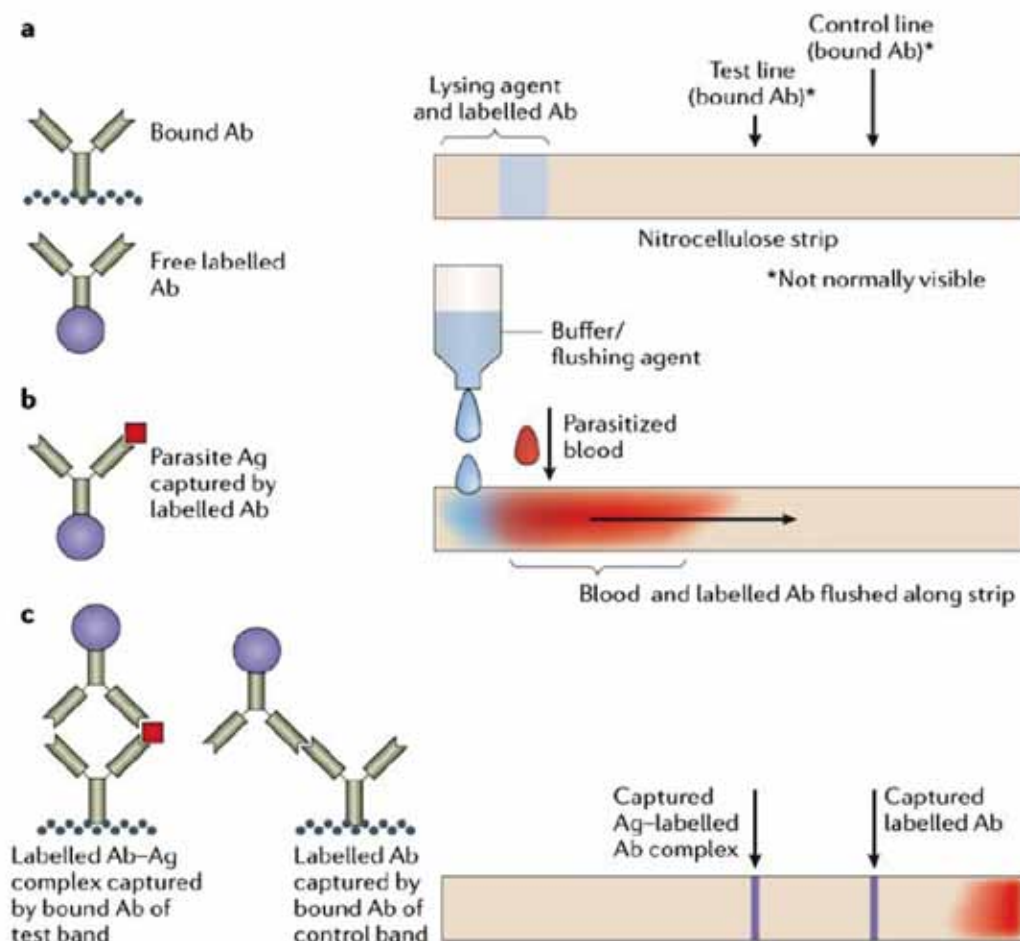


Figure 7.3. Mode of action of antigen-detecting malaria rapid diagnostic tests (RDTs)
(From Bell D, Wongsrichanalai C, Barnwell JW. Ensuring quality and access for malaria diagnosis: how can it be achieved? *Nat Rev Microbiol.* 2006 4(9 Suppl):S7-20.)

obtained from a finger prick, in a similar way to that usually used for malaria microscopy. A small sample of blood, usually 5 to 20 μL , is placed on the RDT strip, or in a well of the cassette or card test device, and lysed to release the Ag from within red blood cells and parasites from within these cells (a variable amount of Ag is also present in the serum). After several minutes, the test produces a series of visible lines to signal the presence or absence of Ag in the blood sample by the mechanism outlined below. (a) Dye-labelled antibody (Ab), specific for the target Ag, is present on the lower end of the nitrocellulose strip, or in a well provided by a casing covering the strip. Ab, specific for another epitope on the target Ag, is bound to the strip in a thin (test) line, and Ab specific for the labelled Ab is bound at the control line; (b) Blood and buffer, which have been placed on the strip or in the well, are mixed with labelled Ab and are drawn up the strip across the lines of bound Ab; (c) If Ag is present, labelled Ab will be trapped on the test line. Other labelled Ab is trapped on the control line. If sufficient labelled Ab accumulates, the dye labels will become visible to the naked eye as a narrow line.

RDTs for malaria detect either *P. falciparum* histidine-rich protein 2 (*Pf*HRP-2), a water soluble protein specific to *P. falciparum*, or parasite lactate dehydrogenase (pLDH) produced by all four *Plasmodium* species. *Pf*HRP-2 is synthesized throughout the asexual life cycle of the parasite and identified as a surface-exposed protein in infected red blood cells. It is also found circulating in the peripheral blood of infected individuals, hence a good target for the diagnosis of *P. falciparum*. HRP-2 based kits however, cannot be used to monitor treatment efficacy as HRP-2 stays in circulation for as long as two weeks after parasite clearance. While pLDH (an intracellular metabolic enzyme produced by both asexual and sexual stages of malaria parasites) does not persist in the blood, it may provide a good indication of parasite clearance following treatment. Current

test kits detect pLDH from all four species of *Plasmodium* and can differentiate *falciparum* from non-*falciparum* species but not between *P. malariae*, *P. vivax*, and *P. ovale*. Newer RDTs developed can detect both *Pf*HRP-2 and pLDH at the same time.

To date, over 50 brands of malaria RDTs are manufactured, and over 150 products are commercially available. RDTs for malaria are easier to perform than the standard microscopy and have great potential to accurately diagnose malaria in endemic areas. Several malaria RDTs have been tested in the field, and good levels of sensitivity have been achieved with parasitemia levels of >100 parasites/ μL blood. However, sensitivity drops when parasitemia is <100 parasites/ μL . Failure to detect cases with very high parasitemias have been reported. Variability in performance of commercially available RDTs in the field have been found to be influenced by several factors such as kit transport and storage conditions (sensitive to extreme temperature and humidity), quality of manufacture, and variability in interpretation of results by laboratory personnel. Generally, HRP-2 based assays demonstrate comparable sensitivity to good quality microscopy, and other factors affecting their performance have been recently investigated. Genetic diversity of *Pf*HRP-2 gene was determined and it was found that the deduced amino acid sequences are highly polymorphic in different isolates. The number and sequence of specific repeats present in *Pf*HRP-2 vary widely; therefore, the epitopes recognized by the monoclonal antibodies specific to HRP also vary between isolates. Additionally, it was found that monoclonal antibodies raised against *Pf*HRP-2 can also bind to *Pf*HRP-3 which raises its potential role in the performance of HRP-based RDTs. Despite extensive global sequence variation in *Pf*HRP-2, no statistically robust correlation between gene structure and RDT detection rate for *P. falciparum* parasites at 200 parasites/ μL blood was identified. However, a more recent

investigation in the Amazon region of Peru found that a large proportion of *P. falciparum* isolates lack *Pf*HRP-2 and *Pf*HRP-3. This finding implies that HRP-2 based RDTs will fail to detect a significant proportion of *P. falciparum* in malaria endemic areas in Peru and should therefore not be used. Instead, pLDH-based RDTs and quality microscopy are recommended for the diagnosis of malaria in the area.

In malaria endemic areas, mixed infections with *P. falciparum* and *P. vivax* are not uncommon; therefore a combination RDT kit is a more appropriate diagnostic method. The Care Start™ Malaria-HRP-2/pLDH (Pf/Pan) Combo Test is a three-band RDT that can detect both *Pf*HRP-2 and pan-pLDH from infected blood. It is a lateral flow antigen detection test in a cassette format. The presence of an HRP-2 line indicates infection with *P. falciparum*, and the presence of a pan-pLDH line indicates infection with one or more of the non-*P. falciparum* species. The presence of both HRP-2 and pan-pLDH lines indicates mixed infection with *P. falciparum* and one or more of the non-*P. falciparum* species. A recent evaluation of this improved RDT against microscopy and PCR-diagnosed blood samples showed good levels of detection for *P. falciparum* and *P. vivax* and poor levels of detection for *P. malariae* and *P. ovale*.

B. RDTs for other parasites

A magnetic immunochromatographic test (MICT) to detect taeniasis caused by the adult worm of the cestode *Taenia solium* and neurocysticercosis caused by the larval forms has been developed based on two specific *T. solium* excretory-secretory proteins, ES33, and ES38. This test detects antibodies against human *T. solium* and can be used as a point-of-care case detection or confirmation. This assay is also a useful tool in identifying tapeworm carriers that must be treated to ensure success of control programs in communities where the

disease is endemic. Evaluation of ES33-MICT showed 94.5% sensitivity and 96% specificity in detecting taeniasis, while ES38-MICT showed 93.9% sensitivity and 98.9% specificity in detecting cysticercosis.

Diagnosis of schistosomiasis relies heavily on stool Kato-Katz technique which can be cumbersome when performing disease surveillance and mapping for large scale control programs. The use of RDT as an alternative method to estimate prevalence and intensity of infection is now undergoing field evaluation. One commercially available RDT to diagnose schistosomiasis detects the presence of circulating cathodic antigen (CCA) in urine. This method eliminates the need for a fecal sample which is more difficult to collect from patients. A positive association between increasing intensity of CCA urine-dipstick test band and fecal egg count was observed; however, difficulty in assigning trace reactions as putative negative or putative positive infection was encountered. Overall diagnostic sensitivity of this CCA urine-dipstick is 87.7% and specificity is 68.1%, a useful supplement to Kato-Katz examination for the rapid detection of intestinal schistosomiasis.

Visceral leishmaniasis is commonly diagnosed by microscopic identification of the parasite in bone marrow, spleen, or lymph node aspirates. In field settings, this method is unsuitable. The development of a rapid diagnostic test using rK39 antigen to detect *Leishmania* antibodies revolutionized the diagnosis of visceral leishmaniasis in the Indian subcontinent. However, the same high level of sensitivity and specificity of the rK39-based RDT cannot be achieved when the test was used in the African subcontinent. To address this issue, another rK38 polyprotein-based RDT was developed which when tested in Sudan and Bangladesh demonstrated a much improved performance than the rK39-based RDT. This new RDT was found to be an excellent serodiagnostic tool and has great potential in

simplifying diagnosis of visceral leishmaniasis at the point-of-care.

C. Towards New and Improved Technologies

Previous experiences in the use of RDTs for malaria diagnosis have documented thermostability as one critical factor affecting variability of their performance in the field. Majority of commercially available RDTs were developed for storage and use at 25–30°C, but are used in malaria endemic areas where ambient temperatures are much higher. As a result, thermolabile reagents such as antigen-detecting antibodies in RDT kits are degraded resulting in poor performance. To overcome these problems, research is currently on-going to find alternative thermostable reagents. The feasibility of using a shark antibody platform to develop diagnostic reagents that are robust in higher ambient temperatures is currently under investigation. Shark antibodies consist of only one variable domain, unlike mammalian antibodies, but they are fully functional. The single domain is a stable polypeptide harboring a full antigen-binding capacity comparable to more common antibody types. Shark antibodies show high target specificity and high affinity for their target. But more importantly, they are extremely stable and could possibly be the ultimate solution to overcome the problems encountered with thermolabile antibodies currently used in many RDTs.

Another promising molecular diagnostic tool in the diagnosis of parasitic diseases is the LAMP assay which is a highly sensitive and specific test that does not require purified DNA (permits diagnosis directly on patient's sample), can be ran in a single temperature (eliminates the need for a thermocycler), allows simultaneous detection of parasites in a single tube when multiplexed (reduces chances of contamination), rapid (results in less than 40 minutes), and results can be read with the naked eye (user friendly and applicable in the field). Recently, parasitologists have adapted this

technology in the diagnosis of several parasites such as *Entamoeba*, *Trypanosoma*, *Taenia*, *Plasmodium*, and *Cryptosporidium*, and current studies show very promising results.

Development of new and improved diagnostics is a fast evolving field; therefore, it is expected that in the very near future, diagnosis of parasitic diseases can be done with ease and confidence at the point-of-care requiring minimal training.

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Quality Assurance in a Parasitology Laboratory

Vicente Y. Belizario, Jr., Winifreda U. de Leon,
Serafin O. Malecosio, Jr., June Rose A. Naig

Quality assurance (QA) refers to a system in which there is a continuous improvement in reliability, efficiency, and utilization of laboratory services. It encompasses all factors that affect laboratory performance such as procedure manuals; quality control for tests reagents, and equipment; workload; work place conditions; training and laboratory staff support. It is an important part of the operations in clinical laboratory practice, which could be attained through:

- A. Internal Quality Control (IQC), in which a set of procedures is utilized by laboratory personnel in the assessment of their laboratory work. Internal quality control allows the laboratory to look at its own processes, ensures that the staff performed the test to the best of their ability with utmost care. It can be done frequently as needed, and it is more economical compared to external quality control.
- B. External Quality Assessment (EQA), in which there is an objective and periodic assessment of the laboratory performance by an outside party or agency. External quality control provides early warning for systematic problems in laboratory processes, indicates areas that need improvement, identifies training needs, provides objective evidence of testing quality, and allows comparison of performance and results among different test sites.

The three types of external quality assessment are:

1. Proficiency testing, in which unknown samples are sent to the laboratory for

testing, and the results are compared to the standard. It gives an objective measure of the laboratory performance and it is cost-effective.

2. Rechecking or retesting, in which the slides that have been read are rechecked or samples that have been analyzed are retested by the reference laboratory. It is useful when it is difficult to prepare samples to test all of the testing process. It is expensive and uses considerable staff time.
3. On-site evaluation is done when it is difficult to conduct traditional proficiency testing or to use the rechecking/retesting method. It is expensive, and requires staff time and travel time.

QA in a diagnostic parasitology laboratory is a guarantee of reliability of the results obtained in the diagnosis of parasitic infections. Its main objective is to make sure that the laboratory produces reliable, relevant, and reproducible results based on generally agreed principles and using accepted criteria. Accurate laboratory diagnosis of parasitic infections provides a sound basis for the provision of appropriate treatment, as well as a basis for formulation of health policy (Figure 7.4).

The components of quality assurance, which are important in producing reliable results, include the proficiency of laboratory personnel and the use of standardized techniques. The standard techniques start from the choice of procedure and reagents, collection of parasitologic samples (stool, urine, blood, orifice swabs, aspirates, etc.) to the proper processing of the specimen, accurate reading, and correct reporting of results.

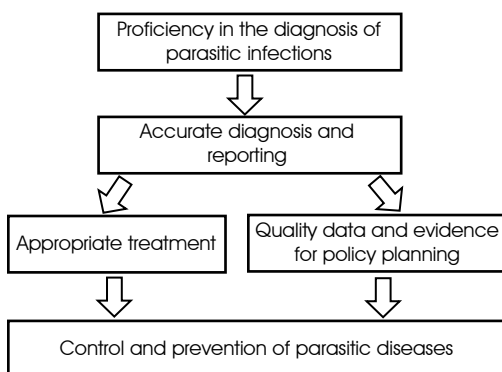


Figure 7.4. A flowchart showing the importance of ensuring quality of laboratory diagnosis of parasitic infections

Quality Assurance Program for Parasitology

A number of quality assurance programs for parasitology have been established in different countries, and one good example is the United Kingdom National External Quality Assurance Scheme (UKNEQAS) for Parasitology. It has raised the level of awareness on parasitic infections in UK laboratory practice by highlighting problem areas and providing focused teaching/training. The UKNEQAS was designed to improve the diagnosis of parasitic disease by examination of samples from patients with parasitic infections, to provide teaching material illustrating unusual or uncommon parasites, and to target areas where a particularly poor performance was noted. At present, the UKNEQAS has the following sub-schemes: (a) fecal parasitology, including extra-intestinal parasites; (b) blood parasitology, including tissue parasites; (c) *Toxoplasma* serology; and (d) the teaching sub-scheme.

In the Philippines, every clinical laboratory is required to have a quality assurance program (QAP) as a requirement for procurement of license. The QAP shall include an Internal and External Quality Assessment Program. In the Internal QAP, implementation of internal quality control measures should be ensured in each laboratory. Laboratory staff, regardless

of the hierarchical status, should strive and be responsible in achieving quality. Current laboratory status should be made, deficiencies identified, and appropriate steps initiated. On the other hand, in the External QAP, a clinical laboratory is required to participate in the National External Quality Assessment Scheme (NEQAS) administered by designated National Reference Laboratories (NRLs). The NEQAS is a schematic quality assessment of laboratory processes using materials of known but undisclosed results through an external agency. It is conducted to ensure that laboratory procedures are done in accordance with standards, and that laboratory results are accurate and within the standard range for quality health care. The Research Institute of Tropical Medicine (RITM) is the reference laboratory designated to conduct NEQAS in parasitology laboratories.

Three Stages of Quality Assurance in a Parasitology Laboratory

The QA in a clinical laboratory encompasses the entirety of the testing process beginning with a clinician ordering a test and ending with the clinician interpreting the results. All activities necessary to produce accurate results are part of quality assurance. It is divided into three stages, namely, pre-analytical, analytical, and post-analytical stage. The pre-analytical stage includes activities performed before the actual laboratory procedure that influence the quality of laboratory results. These activities include: the training of personnel conducting the test; preparation of a patient before specimen collection; specimen collection; specimen quality and volume; and specimen handling and labeling. The analytical stage includes technical or laboratory procedures performed to produce accurate test results. It covers routine work organization, the type of test and reagents, the state of the equipment, and standard operating procedures. It also includes all aspects of quality control including corrective measures to be done

when inaccuracies in the results are identified. The post-analytical stage includes proper and accurate reporting of results. It includes organization of recording, reporting, and interpretation of results, and speed of reporting.

Personnel

The laboratory supervisor has overall responsibility for QA in a diagnostic parasitology laboratory. The qualifications of the supervisor must be consistent with the existing policies on the operation of a clinical laboratory. The supervisor should ensure that:

1. A procedure manual is available
2. Records are properly kept
3. Controls are available for diagnostic procedures
4. Equipment and instruments (e.g., microscopes, incubators, centrifuges, etc.) are properly functioning and calibrated
5. Clerical and analytical errors (if committed) are corrected
6. Unusual laboratory results (e.g., uncommon parasites, unusual antibody titers, etc.) are checked
7. Standardized procedures are being followed in the laboratory

All laboratory personnel should be trained on the different aspects of running a parasitology laboratory. The person in charge of providing instructions to the patient must be familiar with all aspects of specimen collection including preparation of the patient, specimen collection times, sample quality and volume, condition of specimen container, use of preservatives, and proper labeling. Laboratory staff must be familiar with the appropriate diagnostic procedures to be used for each type of specimen and parasite, and must be competent in morphologic recognition and differentiation of parasites.

Procedure Manual

A procedure manual must be made available to the laboratory personnel for reference. This should contain the following information:

1. Instructions for proper collection and handling of samples
2. Information on when to reject parasitologic sample (e.g., inadequate amount of specimen, specimen not labeled with full name or ID number, improperly preserved specimen, etc.)
3. Preparation of reagents and solutions
4. Detailed description of techniques
5. Criteria for identification of parasites
6. Quality control procedures
7. Reporting and interpretation of results
8. General safety precautions (e.g., use of gloves, laboratory gown, proper disposal of specimens, and proper handling of inflammable and hazardous reagents, etc.)

In a study that assessed the quality assurance of a number of clinical laboratories in Iloilo, Philippines, 65.5% of laboratories visited were shown not to have any manual of procedures available as reference.

Instruments and Equipment

A diagnostic parasitology laboratory must be adequately equipped in order to guarantee efficiency. As in any laboratory, preventive maintenance of instruments and equipment must be routinely done. This will ensure that all instruments and equipment are in good condition and are properly functioning.

The most important instrument used in a diagnostic parasitology laboratory is the microscope. It needs constant care to keep it in good working condition. The alignment of the condenser must be regularly checked. The microscope must be protected from dust,

vibration, and moisture. Heat and humidity can lead to fungal growth, which can damage the lenses. The lenses should be cleaned regularly using lens tissue and not other types of tissues that may scratch the lenses. Desiccants should be placed in the microscope cabinets to prevent accumulation of moisture.

In the identification of protozoan cysts in particular, size is taken into consideration. It is recommended that microscopes should be calibrated using an ocular and a stage micrometer.

A stereoscopic microscope should also be available in a diagnostic parasitology laboratory for easier examination of large specimens such as adult worms and worm segments, as well as arthropods.

In most concentration techniques, centrifugation is necessary. Centrifuges must also be calibrated for appropriate speed. Properly calibrated balancing tubes at opposite buckets are strongly recommended to prevent damage to the centrifuge and breakage of test tubes. The inner walls of the centrifuge must be wiped with antiseptic after each use.

Temperature of refrigerators, incubators, water baths, and freezers must be regularly checked using a standard thermometer. A good diagnostic parasitology laboratory should also have a fume hood where the use of volatile or toxic chemicals for diagnostic procedures such as ether and formalin should be done.

Additional items that may be needed in a parasitology laboratory include pH meter, differential counter, and glassware (e.g., microscope slides, volumetric flasks, beakers, funnels, drop bottles, pipettes, test tubes). Chipped glassware must be properly discarded. With the increasing problem on parenterally-transmitted organisms (e.g., Hepatitis B virus, HIV, malaria), the use of disposables like gloves, syringes, and needles is also recommended.

Reagents

In practice, not all reagents in a parasitology laboratory require periodic review; however, antigens, stains, and fixatives should be checked prior to use. Reagents for concentration techniques like zinc sulfate solution may require checking of specific gravity, while the pH of buffer reagents may also have to be checked. All reagents must be properly labeled, and must have preparation and expiration dates. It is also wise to remember which reagents must be kept in sealed containers or dark bottles to prevent degradation. Special precautions must be observed in handling and storing of explosive chemicals like phenol crystals, as well as flammable solvents like xylene, ether, and acetone.

If reagents are purchased commercially, lot and/or batch numbers must be properly noted. Overstocking of commercially prepared staining solutions for blood parasites must be avoided. The quality of the stain can be checked by using a positive slide as control at least once with every new batch of stain. The use of control reagents is strongly recommended particularly in seroimmunodiagnosis.

Appropriate Parasitologic Techniques

The use of appropriate parasitologic techniques in the laboratory will help ensure accuracy of results from diagnostic procedures. The choice of laboratory technique may depend on what is being considered as part of differential diagnosis and what particular purpose the laboratory examination is being requested for. For instance, diarrheic stools that might contain *Entamoeba histolytica* or *Giardia duodenalis* may be best examined using direct fecal smear. A number of intestinal helminths are better demonstrated using modified Kato thick smear method than by direct fecal smear. Routine parasitologic screening and diagnosis

in health centers and hospitals may make use of combined direct fecal smear and modified Kato thick smear method to increase the chances of catching true intestinal parasitic infections. Food handlers are best screened using formalin-ether/ethyl acetate concentration technique (FECT) than by direct fecal smear or modified Kato thick smear method. Modified Kinyoun stain may better demonstrate intestinal coccidian infections. A clinical trial using new anthelmintics against soil-transmitted helminth or schistosome infections benefits most from the use of Kato-Katz method that allows quantitative diagnosis in terms of egg counts. Control programs for soil-transmitted helminth and schistosome infections may utilize Kato-Katz method to demonstrate baseline and follow-up parasitologic parameters (Table 7.6).

All the health center laboratories in Iloilo, Philippines utilize direct fecal smear (DFS) technique for parasitological diagnosis, while only 3.7% of health center laboratories made use of Kato thick smear method, Kato-Katz method or FECT.

Table 7.6. Recommended stool examination techniques for specific situations

Specific situation	Recommended stool examination technique
Routine clinic/hospital stool examination	Direct fecal smear and modified Kato thick method
Examination of diarrheic stools	Direct fecal smear
Screening of food handlers	Formalin-ether/ethyl acetate concentration technique
Screening of Overseas Filipino Workers	Formalin-ether/ethyl acetate concentration technique
Soil-transmitted helminthiasis / schistosomiasis surveillance	Kato-Katz technique
Epidemiologic investigations	Direct fecal smear, modified Kato thick method, Kato-Katz technique, and formalin-ether/ethyl acetate concentration technique (depending on the parasitic infections being investigated)

Reporting

Results of an examination done in the laboratory must be regarded as confidential information between the laboratory and the requesting physician. All laboratory requests together with the results must be properly kept in a logbook or a worksheet which can only be accessed by authorized personnel. The date of examination and reporting of results should also be properly recorded. Qualifying statements regarding the quality of specimen such as “inadequately preserved when received in the laboratory” or “contaminated with water and urine” should also be noted.

In the reporting of results, the complete name (i.e., genus and species) of the parasite must be mentioned and specific stage/s (e.g., ova, larvae, adults, cysts, trophozoites, etc.) must also be indicated. The presence of Charcot-Leyden crystals (CLCs) and budding yeast cells must be noted. CLCs indicate an eosinophil response and are usually associated with allergic or parasitic disease. There is no common convention for the reporting units of CLCs. It is reasonable to report them on presence/absence basis only. Likewise, the presence of macrophages, eosinophils, and polymorphonuclears is important information for a clinician. Quantitative reporting of fecal leukocytes per high power field is recommended (WBC/HPF). However, caution must be taken because fecal leukocytes could be mistaken for amebae.

Pitfalls in the Diagnosis of Parasitic Infections

Laboratory diagnosis of parasitic infections plays an important role in the early detection of disease. In the Philippines, the diagnosis of parasitic infections is still much dependent on the ova and parasite (O and P) examinations. In some instances, artifacts such as fungal spore, mite egg, plant cell, and pollen grain are mistaken as parasite ova. Other artifacts like plant hair can be confused as helminth larvae. Howell-Jolly bodies and nucleated red blood

cells are sometimes misidentified as malaria parasites. Fungal spores of *Helicosporium* may also be mistaken as microfilariae (Plates

7.8–7.15). These practices lead to inaccurate diagnosis of parasitic infections (false positives) and inappropriate treatment of patients.



Plate 7.8. A fungal spore in a wet mount stool may look like a cyst of *Entamoeba* spp.
(Accessed from www.dpd.cdc.gov/dpdx)



Plate 7.9. A mite egg in a formalin-concentrated stool specimen may look like a hookworm egg.
(Accessed from www.dpd.cdc.gov/dpdx)

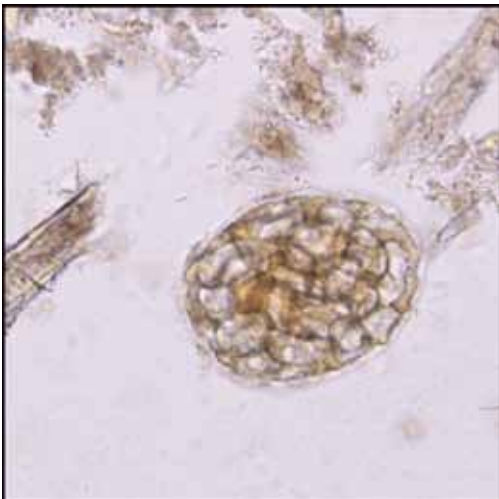


Plate 7.10. A plant cell in a concentrated wet mount of stool may look like a helminth egg.
(Accessed from www.dpd.cdc.gov/dpdx)

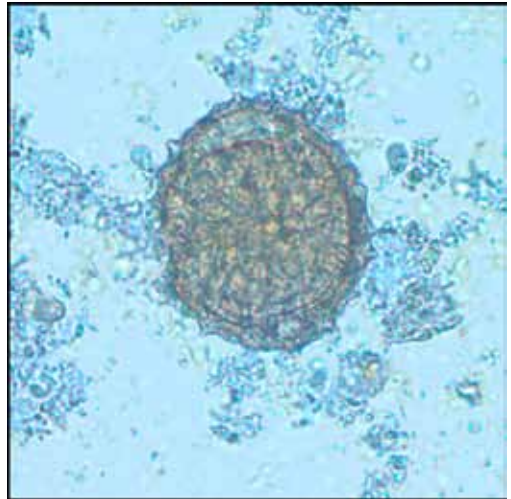


Plate 7.11. A pollen grain in a concentrated wet mount of stool may look like a fertilized egg of *Ascaris lumbricoides*.
(Accessed from www.dpd.cdc.gov/dpdx)



Plate 7.12. Plant hair in a concentrated wet mount of stool may look like a hookworm or *Strongyloides stercoralis* larva.
(Accessed from www.dpd.cdc.gov/dpdx)

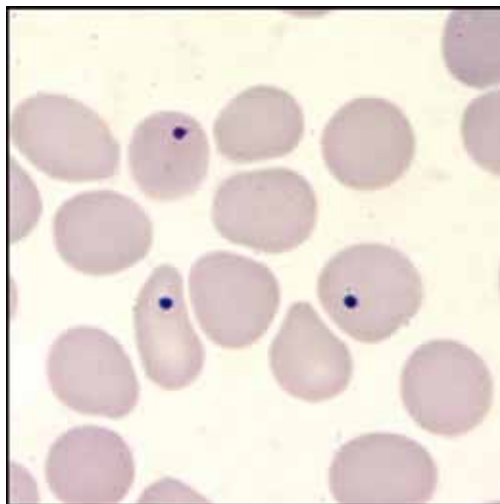


Plate 7.13. Howell-Jolly bodies in a thin blood smear stained with Giemsa may look like malaria parasites.
(Accessed from www.dpd.cdc.gov/dpdx)

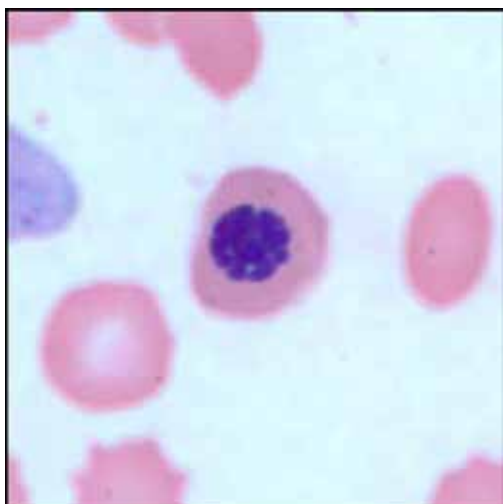


Plate 7.14. A nucleated red blood cell may look like a schizont of *Plasmodium* spp.
(Accessed from www.dpd.cdc.gov/dpdx)

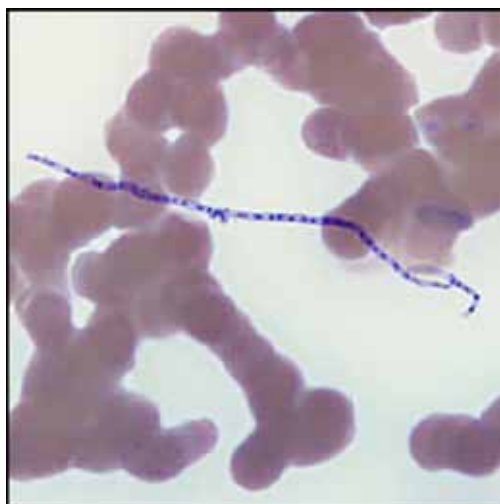


Plate 7.15. Fungal spores of *Helicospirium* may be mistaken as microfilariae in stained blood smears.
(Accessed from www.dpd.cdc.gov/dpdx)

Quality Assurance in Parasite Microscopy

For many parasitic conditions as in helminth infections and malaria, microscopic examination is still considered to be the “gold standard” procedure. Microscopists, however,

differ in terms of basic training and skills. A physician, health manager, or a scientific investigator should be assured of the quality of the microscopic examination. On some occasions, the requesting party may also be interested to know the burden of infection

of the patient. This can be determined by counting the number of parasite or parasite ova in a predetermined amount of sample, and reporting the intensity as number of parasite/ova per volume or weight of sample. In practice, quantification of malarial parasites in blood is more commonly requested. The result is reported as the number of malaria parasites per microliter of blood. Quantifying the parasites also helps in the assessment of the efficacy of interventions (e.g., chemotherapy) in the control of specific parasitic infections.

The reliability of results depends on the ability of the microscopists to identify and count stages of the parasites. This can be assured by cross-checking of the same samples, whereby the reading of the initial microscopist is compared to the reading of a reference microscopist. The reference microscopist must be “blinded” or must have no prior knowledge of the reading of the initial microscopist.

For malaria microscopy, quality control may be done in a laboratory through “blinded” cross-checking of a minimum of 10 randomly selected slides (five reported as low-density, five reported as negative). This is done by a trained validator/cross-checker at the end of each month. When the number of tests performed in one month is less than the minimum sample size, all slides must be cross-checked.

For the soil-transmitted helminth infections and schistosomiasis, all negative slides and 10% of the positive slides may be reread by a reference microscopist. The main drawback of this procedure is that the technique may be laborious and not be feasible in many settings, such as in large-scale examination in surveillance and monitoring of the impact of control programs.

It has been difficult to maintain good quality for malaria microscopy especially in peripheral laboratories. Current challenges in malaria microscopy include:

- Lack of political commitment to support the development and expansion of laboratory services

- Poor quality of microscopy, particularly at the peripheral level
- Difficulties in maintaining microscopy facilities in good order
- Logistic problems and high costs of maintaining adequate supplies and equipment
- Lack of adequate training and retraining of laboratory staff
- Delays in providing results to clinical staff
- Lack of quality assurance and supervision of laboratory services
- Inability to cope with the workload of traditional systems for cross-checking of routinely taken malaria slides

These limitations can only be overcome by new health policies that acknowledge the importance of strengthening laboratory services, the need for adequate funding, and the implementation of a QA system. Such policies should ensure the following:

- Adequate staff and resources
- Regular training and supervision of staff, and quality control of their tasks
- Accurate and timely slide collection, staining, and reading, linked to clinical diagnosis
- Reliable results quickly provided to clinicians
- Provision of logistic support for quality supplies and equipment

At present, a QA program designed by the World Health Organization is used to assist managers of national malaria control programs and laboratory services to develop and maintain a sustainable malaria microscopy QA program. This program outlines a hierarchical structure based on re-training, validation, and the development of competency standards designed to ensure the quality of diagnosis necessary for a successful malaria program.

When validation of results is not feasible through slide cross-checking, a certification

scheme for microscopists may be considered. Microscopists will initially undergo training to enhance their basic knowledge on the parasites. An intensive training on microscopic diagnosis of parasitic infections will provide basic competency, which will be assessed through theoretical and practical examinations, the latter using prepared control slides. Sensitivity and specificity scores of the microscopists will be determined. A certificate will then be awarded to the microscopists who passed the theoretical and the practical examinations administered by a panel of experts. After certification, possible questions on the veracity of results issued by the microscopist may be minimized. The certified microscopists will also develop a higher level of confidence in performance of their laboratory work.

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Special Topics in Parasitology

Parasitic Zoonoses

Salcedo L. Eduardo

Zoonoses as defined by the World Health Organization (WHO) are “those diseases and infections which are naturally transmitted between vertebrate animals and humans.” Zoonotic parasites therefore are protozoan, helminth, and arthropod agents transmitted between these mentioned hosts.

Zoonoses include a wide assemblage of diseases with varied epidemiological and clinical features. Thus, attempts have been made for their groupings. Zoonoses have been classified based on reservoir host of the causative agent, whether humans or lower vertebrate animals. Those that are transmitted: to humans from lower vertebrates are *anthropozoonoses*, to lower vertebrates from humans are *zooanthropozoonoses*, and in either direction and maintained in both humans and lower vertebrates are *amphixenoses*. This classification however has caused confusion and sometimes, has been used indiscriminately.

A more acceptable classification is that of Schwabe in 1984, which is based on the life cycle of the etiologic agent. The zoonoses are grouped as follows: (a) direct zoonoses, (b) cyclozoonoses, (c) metazoonoses, and (d) zaproozoonoses. This classification is convenient for use for teaching purposes since prevention

and control measures of the causative agent are dependent on the knowledge of its life cycle. The groupings are presented in more detail with examples and illustrations in succeeding parts of this section.

Parasitic Zoonoses

Only zoonotic agents that have been reported in the Philippines, either in humans or in animals or both, are considered in this section. These are arranged according to the life cycle of the agent. As majority of these are already presented in detail in other sections of this book, the information given here are those of the animal hosts and the mode of transmission to humans. The assignment of some to a particular group (e.g., intestinal capillariasis) is temporary until the life cycle in nature is exactly known.

A. Direct Zoonoses

In this group are those infections that are transmitted from an infected vertebrate host to another vertebrate host by contact, fomite, or mechanical vector with little or no developmental change in the causative agent during transmission (Figure 8.1).

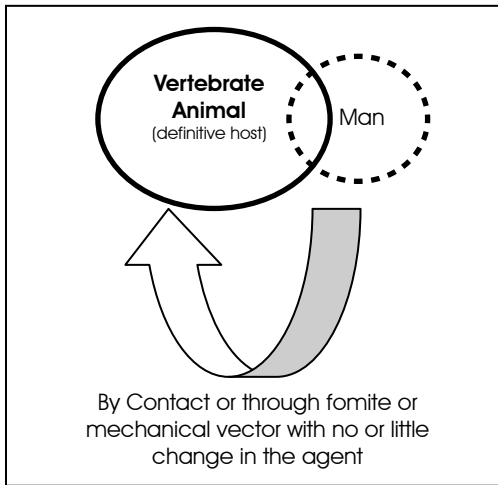


Figure 8.1. Direct zoonoses

1. *Balantidiasis*

The causative agent of this disease is *Balantidium coli* (Plate 8.1), which is a cosmopolitan parasite of pigs. Infection occurs when cysts from feces of infected animals are ingested through contaminated food and water. Human infection is sporadic but common among workers in piggery establishments.



Plate 8.1. *Balantidium coli* from pig
(Courtesy of Dr. Salcedo Eduardo)

2. *Cryptosporidiosis*

A number of species of the genus *Cryptosporidium* has been recorded but only *Cryptosporidium parvum* is known to cause zoonotic infections. This species has been recorded in a wide range of domestic and wild animal hosts. It is one of the causes of diarrhea in lambs and calves. In the Philippines, *Cryptosporidium* has been recorded in cattle, water buffaloes, pigs, and chickens.

Human infection results from ingestion of infective oocyst through contaminated food and water from infected persons or animals. Infection is usually severe in immunocompromised persons. Human cases of cryptosporidiosis have been recorded in the Philippines both in urban and rural areas and in diarrheic and cancer patients, with a prevalence rate of 1.9% in the latter.

3. *Amebiasis*

The disease is caused by *Entamoeba histolytica* and human is its principal host. It is however widespread in non-human primates. It is cosmopolitan in distribution but more common in the tropics and subtropics, especially in areas with low economic status where poor hygienic conditions occur and favor transmission. Food, especially raw vegetables and fruits, as well as water contaminated with cysts from feces are important sources of infection. Transmission through infected food handlers also exists.

4. *Giardiasis*

More than 50 species of the genus *Giardia* have been described but only five are currently recognized as distinct, including *Giardia duodenalis* (also known as *Giardia intestinalis* and *G. lamblia*). *G. duodenalis* affects human and a wide host range of animals including numerous mammalian species. *Giardia* is highly prevalent in domesticated animals. Fecal-oral transmission is common especially among inmates in institutions and prisons,

and in animals in close confinement aggravated by coprophagy. Humans become infected by ingesting cysts directly through fecal-oral transmission or contaminated food or water.

5. *Scabies (human) and mange (animals)*

Aside from *Sarcoptes scabiei*, *Sarcoptes* spp., and *Demodex* spp. from other animals, as well as *Notoedres cati* of cats affect humans. These mites are the cause of mange in animals. Lesions are usually found on the hands and forearms of pet owners. *Sarcoptes* spp. from cattle, water buffaloes, horses, pigs, and *S. scabiei* of human and *Notoedres cati* of cats have been recorded in the Philippines.

6. *Trombiculidosis*

The mite family Trombiculidae (chigger mites) is an assemblage of several genera whose adults and nymphal stages are free living but whose larvae normally attack rodents, insectivores, and ground dwelling birds causing dermatoses. Given the opportunity, they will feed on human, livestock, and poultry. In the Philippines, the following trombiculid genera have been recorded: *Eutrombicula*, *Leptotrombidium*, *Neoschoengastia*, *Schoengastiella*, *Trombicula*, *Toritrombicula*, and *Walchiella*.

Eutrombicula wichni is the cause of human trombiculidosis. *Leptotrombidium akamushi* is a known vector of scrub typhus or tsutsugamushi disease in humans caused by *Orientia tsutsugamushi*. Scrub typhus has been reported in China, Japan, Southwest Pacific to Siberia, and Pakistan. Cases of human infestation in the Philippines have occurred especially among soldiers during World War II and cases of infestation in the navel and scrotum among children playing on areas where rat nests abound.

B. Cyclozoonoses

To this group belong those infections whose causative agents require only vertebrate hosts

(one definitive and the other intermediate hosts) and no invertebrate hosts in the completion of the life cycle. Humans may be obligatory or non-obligatory hosts. There are two subtypes.

Subtype 1: Human as an obligatory definitive host (Figure 8.2)

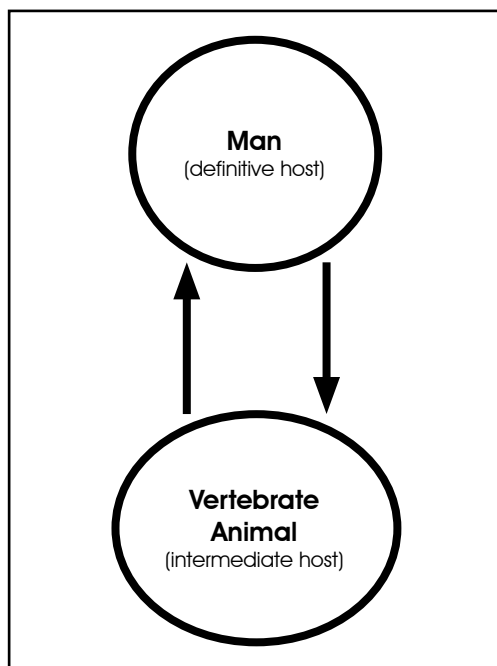


Figure 8.2. Cyclozoonoses subtype 1: human as an obligatory (definitive) host

1. *Sarcocystosis*

Members of the genus, *Sarcocystis*, which has an obligatory prey-predator two-host cycle, cause this condition. Asexual and sexual stages develop in the intermediate and definitive hosts, respectively. Intermediate host becomes infected through ingestion of oocysts in food and water contaminated with feces of the definitive host. Definitive host becomes infected through ingestion of mature sarcocysts (Plate 8.2) from tissues of infected intermediate host.

Humans serve as definitive hosts for two species, namely, *Sarcocystis hominis* and *S. suihominis*. Cattle and swine serve as the

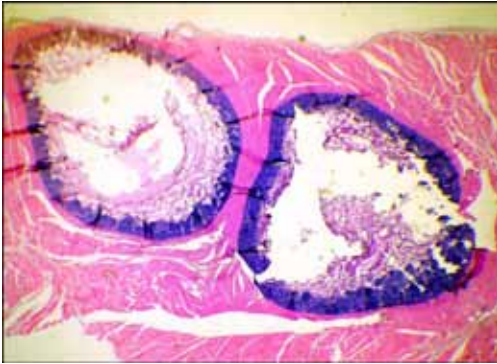


Plate 8.2. Sarcocyst in sectioned esophageal muscle of water buffalo
(Courtesy of Dr. Salcedo Eduardo)

intermediate hosts, respectively. Humans can also serve as accidental intermediate hosts of a number of species occurring in animals. *Sarcocystis* species have been reported from the muscles of cattle (*S. cruzi*), water buffaloes (*S. fusiformis*, *S. levinei*), goats (*S. capracanis*), and swine (*S. mieschieriana*) in the Philippines.

2. Taeniasis/Cysticercosis

Taenia solium and *T. saginata asiatica* are the causes of human taeniasis in the Philippines. What was previously referred to in literature as *Taenia saginata* or *Taenia saginata*-like in Taiwan, Korea, China, Thailand, Indonesia, and the Philippines is in fact *Taenia saginata asiatica*. *Taenia solium* requires pig as the intermediate host and the cysticerci (Plate 8.3) are found in the host's muscles. The true *Taenia saginata* requires cattle and water buffaloes as intermediate host and its cysticerci are also found in the muscles. *Taenia saginata asiatica*, on the other hand, requires pig as the intermediate host and its cysticerci occur mainly in the liver. Examination of slaughtered pigs during meat inspection should therefore include the liver and not only the muscles as is currently practiced. Between the two species, *T. saginata asiatica* is more common than *T. solium*. The former species is endemic in Leyte with a prevalence of 10%.



Plate 8.3. Cysticercus cellulosae freed from muscle of pig
(Courtesy of Dr. Salcedo Eduardo)

Human cases of cysticercosis have been reported in the Philippines and were diagnosed to be, or highly suggestive of *T. solium*. In animals, cysticercosis is more common in swine (1.67%) than in cattle (0.02%) or water buffalo (0.03%).

Another species that can cause taeniasis in humans is *Taenia taeniaeformis*, a common intestinal tapeworm of cats. Rodents and rabbits serve as intermediate hosts for the larval stage called *Strobilocercus fasciolaris* (Plate 8.4), which is found in their liver. In the Philippines, the prevalence in rats may range from 21.4% to as high as 97.0%. Rice field



Plate 8.4. *Strobilocercus fasciolaris* freed from liver of field rat
(Courtesy of Dr. Salcedo Eduardo)

rats (*Rattus tanezumi*) examined in Bay, Laguna revealed 37.4% infection with *S. fasciolaris* (unpublished). Cats become infected through ingestion of infected rat liver where the larva is released in the intestine, attaches to the mucosa to grow to maturity. Similarly, infection in human can result from ingestion of raw or improperly cooked liver of infected rodents. Although human infections with this species have been reported in other parts of the world, none has been recorded in the Philippines.

Subtype 2: Human as a non-obligatory (optional) host (Figure 8.3)

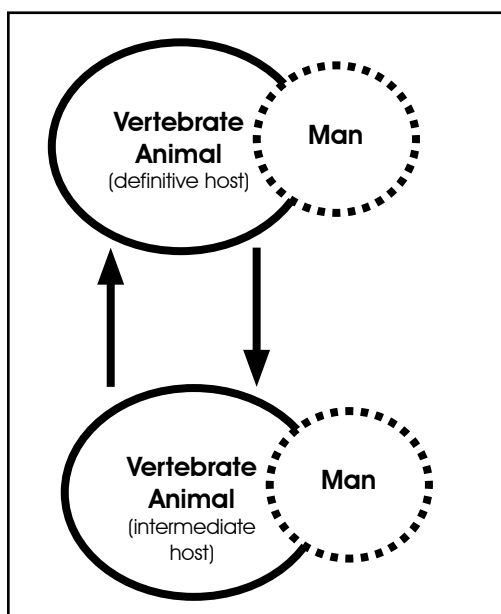


Figure 8.3. Cyclozoonoses subtype 2: human as a non-obligatory (optional) host

1. Toxoplasmosis

This disease, caused by *Toxoplasma gondii*, is widespread among warm-blooded animals, including humans. Cats and other wild felids serve as the definitive hosts. Transmission occurs through the placenta and ingestion of the infective forms found encysted in tissues (bradyzoites or cystozoites) of infected animals or in cat feces (sporulated oocysts) by way of

contaminated food and water. Transplacental infection occurs when previously non-infected hosts become infected during pregnancy. The organism multiplies in the placenta and spreads to the fetal tissues. Cats play an important role in the transmission of *T. gondii*. The disease is a major public health concern because of the risk of transplacental transmission when cats (as the source of infective oocysts) are in the same households with pregnant women. Oocysts are resistant to most disinfectants and can survive up to two and a half years even in unfavorable environmental conditions. In the Philippines, serological surveys revealed prevalence rates to be as high as 52.7% in cats, and 19.0%, 8.1%, 1.9%, and 2.4%, in pigs, rats, water buffaloes, and humans, respectively.

2. Echinococcosis/Hydatidosis

The species involved is *Echinococcus granulosus*. The dog and wild canids are the definitive hosts where the adults occur in the intestine. Mammals, including humans, serve as the intermediate host where the metacestode (*Echinococcus* or hydatid cyst) develops. Humans become infected by ingestion of the egg from infected definitive hosts.

While the disease is common in other parts of Asia, there are only very few reports in the Philippines. There is only one record of *E. granulosus* in a dog, one report of *Echinococcus* cyst in water buffalo, and a few cases of human hydatidosis.

3. Anisakiasis

This condition is caused by the larval stages of anisakine nematodes persisting in the alimentary canal or penetrating the tissues of humans after consuming raw or semi-raw infected fish. A variety of fish species acts as intermediate/transport hosts for the larva (Plate 8.5), which mature to adult in warm-blooded marine mammals. Human cases of anisakiasis have been reported in the Americas, Europe, and Japan. Each year, 1,000 cases are



Plate 8.5. *Anisakis* larva from fish
(Courtesy of Dr. Salcedo Eduardo)

reported in Japan where consumption of raw fish is common. While no human case has been reported so far in the Philippines, a wide range of fish species have been found to harbor anisakine larvae (Table 8.1). The potential of human infection in the Philippines therefore, is great especially now that many Japanese food preparations of raw and semi-raw fish (e.g.,

sushi, sashimi, etc.) are gaining acceptance among Filipinos. It is possible that there may have been unreported human cases of anisakiasis in the Philippines due to lack of awareness by health workers.

4. Capillariasis

The cycle of *C. philippinensis* in nature has not been fully determined. Experimental evidence, however, points to fresh and brackish water fishes as the sources of infection. *Hypseleotris bipartita* has been found to be a natural source of infection in Ilocos Sur. Other species of fish, namely, *Chonophorus melanocephalus* (*bukto*, *biyang bato*), *Ambassis miops* (*bagsang*), *Eleotris melanosoma* (*birut*), *Sicyopterus* sp. (*ipon*), and *Poecilia reticulata* (guppy), found in endemic areas in Northern Luzon, have been successfully infected experimentally. With the exception of *P. reticulata*, the rest are often eaten uncooked and gravid *H. bipartita* is especially relished in the raw state. Human infection may result from consumption of raw or semi-raw fish infected with larvae.

Table 8.1. Philippine fishes found harboring anisakine larvae (from various authors)

Scientific name (Local name)		
<i>Acanthopagrus berda</i> (<i>bakoko</i>)	<i>Leionathus</i> sp. (<i>sapsap</i> , <i>tambong</i>)	<i>Rastrelliger brachysoma</i> , <i>R. kanagurta</i> (<i>alumahan</i>)
<i>Alectis</i> sp. (<i>pampanong puti</i>)	<i>Lutjanus malabaricus</i> (<i>maya-maya</i>)	<i>Sardinella abella</i> (<i>bagasbas</i>)
<i>Amblygaster sirm</i> (<i>tonsoy</i>)	<i>Lutjanus vita</i> (<i>dayang-dayang</i>)	<i>Sardinella longiceps</i> (<i>tamban</i>)
<i>Apogon ellioti</i> (<i>dangat</i>)	<i>Megalaspis cordyla</i> (<i>oriles</i>)	<i>Saurida tumbil</i> (<i>kalaso</i>)
<i>Caesio lunaris</i> (<i>dalagang bukid</i>)	<i>Mene maculate</i> (<i>hiwas</i>)	<i>Scatophagus argus</i> (<i>kalaso</i>)
<i>Carangoides armatus</i> (<i>lawayan</i>)	<i>Muraenesox cinereus</i> (<i>pindanga</i>)	<i>Scomberomorus commerson</i> (<i>tanigue</i>)
<i>Caranx</i> sp. (<i>talakitok</i>)	<i>Nemipterus</i> sp. (<i>bisugo</i>)	<i>Selar crumenophthalmus</i> (<i>matang-baka</i>)
<i>Decapterus</i> sp. (<i>galunggong</i>)	<i>Otolithes ruber</i> (<i>alakaak</i>)	<i>Selaroides leptolepis</i> (<i>salay salay</i>)
<i>Eleutheronema tetradactylum</i> (<i>mamali</i>)	<i>Oxyurichthys microlepis</i> (<i>talimusak</i>)	<i>Siganus</i> sp. (<i>samaral</i>)
<i>Epinephelus</i> sp. (<i>lapu-lapu</i>)	<i>Pennahia aenea</i> (<i>alakaak</i>)	<i>Stolephorus</i> sp. (<i>dilis</i>)
<i>Euthynnus affinis</i> (<i>kutsarita</i>)	<i>Pinjalo pinjalo</i> (<i>sulid</i>)	<i>Sypnatura sorsogonensis</i> (<i>dapa</i>)
<i>Gerres filamentosus</i> (<i>malakapas</i>)	<i>Poecilia latipinna</i> (<i>bubuntis</i>)	<i>Sphyræna langsar</i> (<i>tursilyo</i>)
<i>Lactarius lactarius</i> (<i>pagapa</i>)	<i>Priacanthus tayenus</i> (<i>bisugong tsina</i>)	<i>Terapon jarbua</i> (<i>bagaong</i>)
<i>Leionathus equulus</i> (<i>lawayakan</i>)	<i>Psettodes erumei</i> (<i>dapa</i>)	<i>Trichiurus lepturus</i> (<i>espada</i>)

Fish-eating birds are believed to be the natural host and are responsible for disseminating infection along their migratory path. Experimental studies showed susceptibility of some birds to infection with the parasite. In the Philippines, *Ixobrychus* sp. (bittern) has been found to harbor a male specimen of the parasite. Other species of fish may be more commonly infected. Autoinfection is a part of the cycle in mammals as evidenced by embryonated eggs and larvae produced by female worms.

C. Metazoonoses

This group includes those infections whose causative agents are transmitted biologically or cyclically by invertebrates, which serve as intermediate hosts and where the agent multiplies. There are three subtypes.

Subtype 1: One vertebrate host (definitive host) and one invertebrate host (intermediate host) (Figure 8.4)

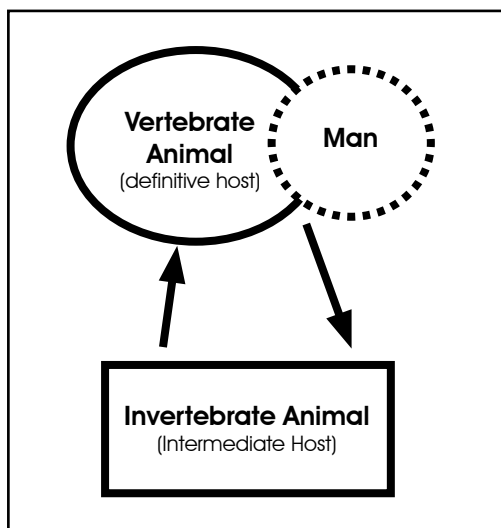


Figure 8.4. Metazoonoses subtype 1: one vertebrate host (definitive) and one invertebrate host (intermediate)

1. Fascioliasis (also a zaprozooses)

Two species, *Fasciola gigantica* and *F. hepatica* (Plate 8.6), have been reported in animals in the



Plate 8.6. *Fasciola gigantica* and *F. hepatica* from water buffalo
(Courtesy of Dr. Salcedo Eduardo)

Philippines but recent investigations based on previous and current collections have shown that the former is the predominant if not the only species now occurring in the country. The prevalence of *Fasciola* infection in domestic ruminants is estimated at 45%, but in endemic areas, it may reach as high as 95%. The snail intermediate hosts in this country are *Lymnaea philippinensis* and *L. auricularia rubiginosa*, which are likewise distributed throughout the islands. Animals become infected through the ingestion of metacercariae (Plate 8.7) encysted on grass and other water plants, or drinking contaminated water.

From literature, cases of human fascioliasis, recognized as an emerging human infection, have been increasing especially in developing

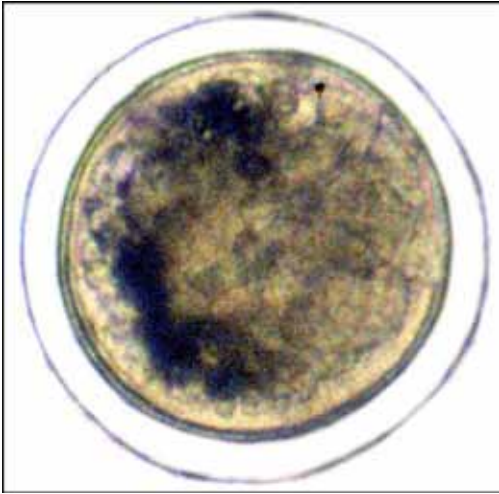


Plate 8.7. *Fasciola* metacercaria
(Courtesy of Dr. Salcedo Eduardo)

countries. It has been estimated that 2.4 million people are infected with this trematode and another 180 million are at risk. In the Philippines, only two cases of human infection with *Fasciola* have been recorded. The exact origin of the infection could not be traced but it probably resulted from the partly cooked edible water plant, *Ipomea* (*kangkong*) or the accidental ingestion of other water plants harboring metacercariae of the fluke. The high prevalence in animals in endemic areas puts the local human population at risk to infection.

2. Schistosomiasis

Schistosoma japonicum is the only species of the genus which causes human schistosomiasis in the Philippines. It is endemic in Sorsogon, Mindoro, Leyte, Samar, Bohol, and Mindanao. The amphibious snail, *Oncomelania quadrasi*, serves as its intermediate host in the country. A variety of domestic and wild animals serves as important reservoir hosts. Recent studies in an endemic area (Samar) in the Philippines, revealed infection in dogs, rats, cats, pigs, and water buffaloes with high prevalence and intensities of infection in dogs and rats. A study in the same province suggested high

levels of transmission between humans and dogs. Infection is through penetration of the skin by the cercaria (Plate 8.8) when the host comes in contact with infected water.

3. Dipylidiasis

Dipylidium caninum (Plate 8.9) infection is common in dogs and cats worldwide. In the Philippines, the prevalence rate especially among stray dogs may range from 5 to 81%. The cat (*Ctenocephalides felis*) and dog flea



Plate 8.8. *Schistosoma* cercaria
(Courtesy of Dr. Salcedo Eduardo)



Plate 8.9. *Dipylidium caninum* from dog
(Courtesy of Dr. Salcedo Eduardo)

(*Ctenocephalides canis*), and the dog louse (*Heterodoxus longitarsus*) serve as intermediate hosts that harbor the cysticeroid stage. Humans, especially children, become infected when fleas and lice containing cysticeroid are accidentally ingested.

4. Hymenolepiasis

Two species with cosmopolitan distribution, *Hymenolepis nana* (previously known as *Vampirolepis nana* and also known as the dwarf tapeworm) and *H. diminuta* are involved in this infection. The adult form occurs in rats and humans. Completion of their life cycle requires intermediate hosts, but for *H. nana*, this is optional. Intermediate hosts include flour beetles, and other arthropods where the metacestode (cysticeroid) is formed. *H. diminuta* is widely distributed among rats with a prevalence rate of 10.8%. In humans, the prevalence is 1%. Human infection results from ingestion of uncooked food contaminated with infected intermediate hosts or their accidental ingestion. The infection is prevalent among children.

5. Raillietiniasis

The genus *Raillietina* is well represented in domestic and wild birds in the Philippines with at least 19 species reported. Only one species, *R. madagascariensis*, also known as *R. garrisoni*, has been involved in human infection, and its prevalence rate among rodents may range from 22% to as high as 86%. It has been shown that beetles and ants serve as intermediate hosts. Human infection results from ingestion of the intermediate host infected with cysticeroid.

6. Parastrongylosis or Eosinophilic Meningoencephalitis

Parastrongylus cantonensis is the cause of the condition and the only species of the genus reported in the Philippines. This species occurs as adults in the lungs of rats (*Rattus* spp.) with prevalence ranging from 3 to 10%.

In the Philippines, land snails (*Achatina fulica*, *Hemiplecta sagittifera*, *Helicostyla macrostoma*, *Chlorea fibula*, and *Cyclophorus* spp.), garden slugs (*Imerina plebeia*, *Laevicaulus alte*) serve as primary intermediate hosts of the parasite. In the rice field, *A. fulica* and other snails have been observed to be important sources of food of rodents and especially when grains become scarce. This snail and infected rats contributed to the spread and its introduction to many regions of the world.

Humans are accidental hosts. Infection results from ingestion of infective larvae frequently through the paratenic hosts (e.g., fresh water prawns) which are eaten raw and whose juices are used in the preparation of local dishes, or ingestion of vegetables contaminated with larvae from infected obligate intermediate hosts. Parastrongylosis in humans affects the central nervous system where the migrating larvae cause a condition called tropical eosinophilic meningitis. Human cases, including cases of ocular parastrongylosis, which are all non-fatal and presumably due to larvae of *P. cantonensis*, have been reported locally.

7. Dirofilariasis/Human Pulmonary Dirofilariasis

Dirofilaria immitis (Plate 8.10), the heartworm of dogs, is common and widely distributed in dogs in the Philippines and transmitted by mosquitoes. Latest data showed an incidence rate of 20% among client owned dogs from the Greater Metropolitan Manila. The dog therefore is an important source for human infection through the bites of infected mosquitoes.

Human infection with this species usually involves the lungs, thus the term pulmonary dirofilariasis. However, the involvement of other organs such as the eye, posterior vena cava, abdominal cavity, spermatic cord, and possibly the meninges has been reported. Although no human case so far has been reported in the Philippines, several cases have been recorded in Australia, Japan, Spain, and the United States.

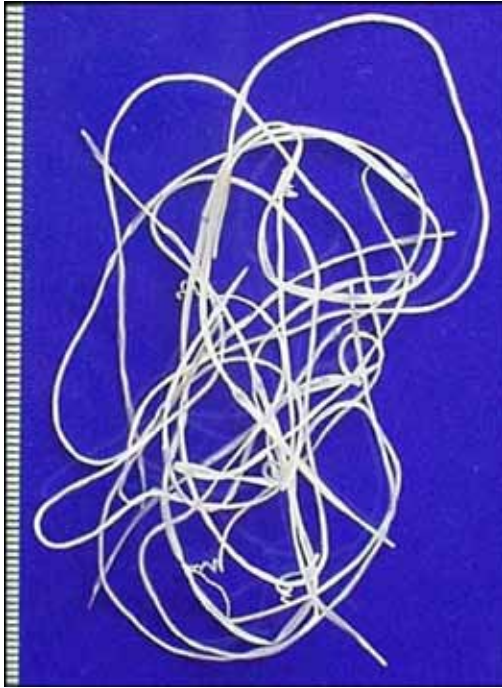


Plate 8.10. *Dirofilaria immitis* from dog
(Courtesy of Dr. Salcedo Eduardo)

8. Cutaneous Parafilariasis in Animals/Ocular Parafilariasis in Humans

Parafilaria bovicola, a nematode, is the causative agent of this condition. First reported in cattle in the Philippines causing hemorrhagic cutaneous lesions, it occurs now in other parts of Asia, Africa, and parts of Europe affecting cattle and water buffaloes (*Bubalus bubalis*). In the Philippines, 3% of water buffaloes have been found infected with this species. Flies of the genus *Musca* spp. have been incriminated as vectors. Flies become infected by feeding on the bleeding sores of infected animals containing eggs or larvae, and eventually develop into the infective larvae. Flies introduce infective larvae to the same or another animal by feeding on wounds or ocular secretions.

The first human infection with this species has recently been reported in Thailand, the worm infecting the eye of a 72-year old man. Infection probably resulted from introduction

of the infective larva by the insect vector feeding on eye secretions.

9. Acanthocephalosis (Macracanthorhynchosis and Moniliformosis)

Two species of acanthocephala of animals can cause this condition in humans. These are *Macracanthorhynchus hirudinaceus* (Plate 8.11) of pigs and *Moniliformis moniliformis* of rats, which are common in the tropics and subtropics. In the Philippines, both species are present. The former is more common among free-roaming backyard-raised pigs than those raised in commercial farms. The latter species is common in both field and laboratory rats. May beetles, *Phyllophaga rugosa*, and cockroaches serve as the intermediate host for the former and the latter, respectively. Cases of human infection have been reported in other countries resulting from accidental ingestion of infected intermediate host, but no case has been recorded so far in the Philippines.



Plate 8.11. *Macracanthorhynchus hirudinaceus* from pig (Courtesy of Dr. Salcedo Eduardo)

Subtype 2: More than one invertebrate hosts (first and second intermediate hosts) and one vertebrate host (Figure 8.5)

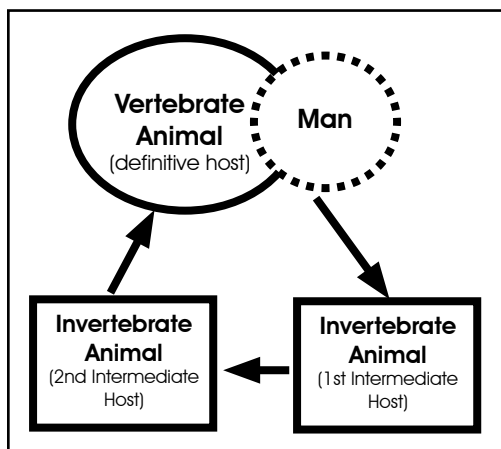


Figure 8.5. Metazoonoses subtype 2: more than one invertebrate host (first and second intermediate hosts) and one vertebrate host

1. Echinostomiasis

Human echinostomiasis in the Philippines is caused by *Echinostoma ilocanum*. The adult fluke is found in the small intestines, thus the disease condition is also called intestinal echinostomiasis. It is widespread with a prevalence of 3%, but it is more commonly found in Northern Luzon where prevalence reached as high as 44%.

Rattus spp. are important animal hosts but dogs and cats may equally be important. However, no data are available on the prevalence of natural infection especially on the last two hosts. A variety of laboratory animals especially rats, mice, and hamsters are the most susceptible experimental hosts.

E. ilocanum requires fresh water snails as intermediate host to complete its life cycle. Locally, the freshwater planorbid snail, *Gyraulus phrasadi* serves as the first intermediate host. The second intermediate hosts include a variety of freshwater snails including *G. phrasadi* and *Pila*

conica. Filipinos eat the latter species, which is considered the primary source of infection. The Ilocanos of Northern Luzon are known to consume partly cooked *Pila conica* (locally called *bisukol*), hence human infection is highest in this region.

Echinostoma lindoense (Plate 8.12) is another cause of human intestinal echinostomiasis. It has been first described and reported as a human infection in Indonesia and later in other Southeast Asian countries (Indonesia, Malaysia, and Thailand) and Brazil in both animals and humans.

It has recently been recorded in rice field rats in the Philippines, although no human case has been reported locally. The life cycle of this species follows the same pattern as that of *Echinostoma ilocanum* and *Artyfechinostomum malayanum*, but in addition, daughter sporocyst is produced. The first intermediate hosts are freshwater snails: *Gyraulus convexiusculus* and *G. sarasinorum*. The second intermediate hosts are snails (*Gyraulus convexiusculus*, *Lymnaea rubiginosa*, *L. exustus*, and *Biomphalaria glabrata*), mussels (*Corbicula lindoensis*, *C. subplanata*), and tadpoles (*Rhacophorus leucomystax*). The intermediate hosts in the Philippines are not yet known but *Gyraulus* and *Lymnaea* abound in the country. Human infection results from ingestion of viable metacercaria contained in the second intermediate hosts.



Plate 8.12.
Echinostoma lindoense from field rat (Courtesy of Dr. Salcedo Eduardo)

2. Artyfechinostomosis

Artyfechinostomum malayanum is the cause of this condition and it is found in the intestines of the infected host. This species is distributed in many East Asian countries, as well as in the Philippines. Pigs, rice field rats, and a monkey have been found naturally infected, and human infections with this species have been reported from Isabela and Tarlac provinces in Luzon, and recently in Siargao Island, Surigao del Norte in Mindanao.

This species requires freshwater snails as intermediate hosts to complete its development. The snails, *Bullastra cumingiana*, *Radix quadrasi*, and *Physastra hungerfordiana* are naturally infected in the Philippines and therefore serve as the second intermediate host. The source of human infection, however, is *B. cumingiana*, which is eaten by some Filipinos. All human cases in Isabela had a history of eating *B. cumingiana*, which is locally known as *birabid*.

3. Carneophalloosis

Carneophallus brevicaeca is the etiologic agent for this condition. In the Philippines, it has been reported in birds (*Sterna albifrons sinensis*), fish (*Glossogobius giuris*), and in humans where it is particularly associated with lesions in the heart and spinal cord. Snails serve as first intermediate hosts, while shrimps (*Macrobrachium* sp.) have been found to harbor metacercariae thus serving as second intermediate hosts for the parasite. Infection occurs through ingestion of raw or partly cooked shrimps. Other invertebrate intermediate and vertebrate definitive hosts still remain to be known.

4. Eurytremiasis

Members of the genus *Eurytrema* are the etiologic agents of this condition which are parasites of ruminants. Four species namely *E. pancreaticum* (Plate 8.13), *E. coelomaticum*, *E. escuderoi*, and *E. ovis* have been recorded in Philippine ruminants (cattle, goats, water



Plate 8.13. *Eurytrema pancreaticum* from cattle
(Courtesy of Dr. Salcedo Eduardo)

buffaloes, sheep). The prevalence of the first three species in cattle and water buffaloes locally is 11.4%, 2.6%, 4%, and 5.3%, 0.66%, 1.33%, respectively. The first three species occur in the pancreas, while the last species in the perirectal fat in sheep. This group requires two intermediate hosts: land snails (first), and grasshoppers and crickets (second). The second intermediate hosts contain the infective metacercarial stage.

Grasshoppers and crickets are among the many insects eaten in many parts of the world. This is especially true in Africa and Asia where these are prepared in a variety of ways as good sources of protein. Human infection results from ingestion of grasshoppers and crickets containing live metacercariae of the *Eurytrema*. Two cases of human infection with *E. pancreaticum* have been recorded in Japan. No human infection with *Eurytrema* so far has been reported in the Philippines.

5. Paragonimiasis

Members of the genus *Paragonimus* are responsible for this condition. In the Philippines, it is *Paragonimus westermani filipinus*. *Paragonimus* infection is endemic in certain areas in the Philippines and most human cases were from Sorsogon, Samar, Leyte, and a few other provinces in Mindanao. Prevalence in endemic areas may reach 4.6 to 12.5%. Rats, dogs, and cats serve as reservoir hosts but the foremost may play an important role in maintaining the cycle in nature. The prevalence in rats may reach 9.4 to 11.1%.

This species requires freshwater snails and crabs as first and second intermediate hosts, respectively, to complete its cycle. Wild boars may serve as paratenic host. In the Philippines, the snails, *Antemelanina asperata* and *A. dactylus*, and the mountain crab, *Sundathelphusa philippina*, serve as first and second intermediate hosts, respectively.

Human infection results from consumption of infected crabs, raw or partly cooked. In endemic areas, inhabitants are known to consume crabs raw. A preparation with fresh crab juice known as *kinagang* is considered a local delicacy in the Bicol Region.

6. Philophthalmosis

Members of the genus *Philophthalmus* are responsible for this condition. Birds and mammals are hosts to a number of species of *Philophthalmus* inhabiting the eyes (conjunctiva under the nictitating membrane) of their host. Three species namely: *Philophthalmus palpebrarum*, *P. gralli*, and *P. luzonensis* have been recorded in avian hosts in the Philippines.

Human infections with *Philophthalmus* have been reported in various parts of the world and the species involved were those found in birds as follows: *P. gralli* (Plate 8.14), *P. lucipetus*, and *P. lacrymosus*. One case of human infection (with *P. gralli*) so far has been recorded in the Philippines. Humans become accidentally infected through the eyes with



Plate 8.14. *Philophthalmus gralli* from duck
(Courtesy of Dr. Salcedo Eduardo)

cercaria in water released by infected snail intermediate host during bathing in areas where these snails abound, or when washing the face with contaminated water.

7. Plagiorchiosis

Plagiorchis species are the causes of this condition especially those occurring in rats, which serve as definitive hosts in nature. The species involved are *Plagiorchis muris*, *P. philippinensis* (Plate 8.15), and *P. potamonides*. All three species have been recorded in rats in the Philippines with prevalence rates of 0.42 to 6.86%, 1.27%, and 6.86 to 14.5%, respectively.

Human infection occurs from accidental ingestion of the second intermediate host (aquatic insects and crustaceans) containing the infective metacercaria. *P. philippinensis* was first reported as infective to humans before it was reported in rice field rats locally. For *P. muris*, cases of human infection have been reported in Japan and Korea, but no human case so far for this species and *P. potamonides* have been reported in the Philippines. The potential risk of human infection with the latter species remains high because its metacercariae have been found



Plate 8.15. *Plagiorchis philippinensis* from rat
(Courtesy of Dr. Salcedo Eduardo)

in the same crab which serve as intermediate host for *Paragonimus westermani* in the country.

Subtype 3: One invertebrate host (2nd intermediate host) and two vertebrate hosts (one definitive host and the other as 1st intermediate host) (Figure 8.6)

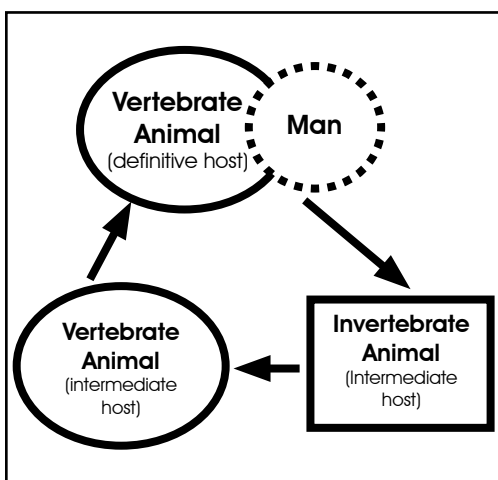


Figure 8.6. Metazoonoses subtype 3: one invertebrate host (intermediate) and two invertebrate hosts (one definitive and one intermediate)

1. Heterophyidiasis

Members of the trematode family Heterophyidae are the causes of this condition. In the Philippines, infection by several species of the family has been recorded in carnivores and birds. Many of these species are known to be transmissible to humans. Four species, namely, *Haplorchis taichui*, *H. yokogawai*, *Procerovum calderoni*, and *Stellanthchasmus falcatus*, also known as *S. pseudocirrata*, have actually been recorded in human infections locally, and these were associated with lesions in the heart, brain, and spinal cord. Unspecified heterophyid infections of humans detected through fecal examination have also been reported.

There are no data available yet on the prevalence of infection in animals. In humans, less than 1% of 3,000 stool samples examined from various places in the country were found positive for heterophyid ova. In Mindanao, however, a high prevalence rate of 36% has been reported for *H. taichui*.

Heterophyids require freshwater snails and fishes as first and second intermediate hosts, respectively, to complete their cycle. A variety of freshwater and marine fishes, have been found infected with the metacercariae of heterophyid species. Philippine fishes found infected with heterophyid metacercariae are enumerated in Table 8.2. Infection therefore occurs when raw or partly cooked fish containing the metacercaria are consumed. The life cycle of only two species, *H. taichui* and *P. calderoni* are known in the Philippines. The snail hosts are *Melania juncea* and *Thiara riquetti*, respectively.

2. Opisthorchiasis

Opisthorchis (Clonorchis) sinensis, the etiologic agent of this condition, has been reported in humans in the Philippines during routine stool examination. In a survey of 30,000 Filipinos, ova similar to that of *Opisthorchis sinensis* were detected in 135 stool samples. This parasite requires snails and a variety of freshwater fishes as intermediate hosts, but for

Table 8.2. Philippine fishes found harboring metacercariae of heterophyid species

Scientific name (local name)	Heterophyid species*	Scientific name (local name)	Heterophyid species*
<i>Acentrogobius janthinopterus</i> (biyang sapa)	PC	<i>Liza subviridis</i> (banak)	HT, HY, PC, SF
<i>Ambassis buruensis</i> (lañgaray)	HY, PC	<i>Mugil dussumieri</i> (tallong)	
<i>Anabas testudineus</i> (martiniko)	PC, SF	<i>Mugil</i> sp. (banak)	HY, PC, SF
<i>Arius manillensis</i> (Manila kanduli)	HY	<i>Oreochromis niloticus</i> (tilapia)	HT
<i>Atherina balabacensis</i> (guno)	PC	<i>Pelates quadrilineatus</i> (agaak)	HY, PC, SF
<i>Butis amboinensis</i> (biyang sunog)	PC	<i>Platycephalus indicus</i> (sunog)	PC
<i>Channa striata</i> (dalag)	HT, HY, PC	<i>Poecilia latipinna</i> (bubuntis)	PC
<i>Chanos chanos</i> (bangus)	PC	<i>Puntius binotatus</i> (paif)	HT
<i>Clarias batrachus</i> (hito)	HY	<i>Rhynchorhamphus georgii</i> (buging)	HY, PC
<i>Eleutheronema tetradactylum</i> (mamali)	PC	<i>Scatophagus argus</i> (kitang)	PC
<i>Epinephelus corallicola</i> (lapu-lapu)	HT, PC	<i>Siganus canaliculatus</i> (barangan)	HY, PC
<i>Gerres filamentosus</i> (malakapas)	PC	<i>Siganus guttatus</i> (barangan)	HT, HY
<i>Gerres kappas</i> (malakapas)	HY	<i>Siganus javus</i> (barangan)	HY, PC
<i>Glossogobius giuris</i> (biya)	PC	<i>Spratellops palata</i> (manobud)	HT
		<i>Terapon jarbua</i> (bagaong)	HT, HY, SF

*Legend: HT-Haplorchis taichui; HY-Haplorchis yokogawai; PC-Procerovum calderoni; SF-Stellantchasmus falcatus

species involved in the Philippines, intermediate hosts are not yet known. Data on animal host infection, especially in carnivores, are still needed. Human infection results from ingestion of live metacercariae from infected fish.

3. Spirometrosis/Sparganosis

Spirometra species and their spargana have been reported in animals in the Philippines. Sparganum (Plate 8.16) is widespread in tadpoles and frogs and has been found in a bird (*Ixobrychus cinnamomeus*), a lizard (*Gecko gecko*), and several species of snake (*Lapemis hardwickii*, *Boiga dendrophila*, *Ahaetulla ahaetulla*, *A. caudolineata*, and *Natrix chrysarga*).

Adult *Diphyllbothrium latum* has been reported locally from a boy who died of anemia. Since *D. latum* is only found in temperate countries of the Northern Hemisphere, this identification is doubtful. It is possible that the species in question is *Spirometra erinacei* or *S. mansonioides*. Stool survey showed diphyllbothrid ova in less than 1% of 30,000



Plate 8.16. Sparganum of *Spirometra* from muscle of frog (Courtesy of Dr. Salcedo Eduardo)

persons examined in various places in the Philippines.

Prior to 1963, only four human cases of sparganosis have been reported locally and since then, no other case has been reported. As all cases gave no history of having eaten fresh meat of frogs, reptiles, and birds, or used them as poultices, the mode of transmission was attributed to the drinking water with infected copepods.

4. Gnathostomiasis

Members of the genus *Gnathostoma* cause this condition. The genus is represented in the Philippines by three species namely, *G. spinigerum*, *G. hispidum*, and *G. doloresi*. All three species have been recorded in humans in other Asian countries but only *G. spinigerum* has been reported in humans locally. *Gnathostoma* spp., in order to complete their development, require aquatic copepods and fishes as intermediate hosts, and a wide range of paratenic hosts may intervene as “extension host” in the cycle.

G. spinigerum has been reported locally in dogs, cats, flying lemurs, and palm civets. Copepods (*Cyclops serrulatus*, *C. bicolor*) and freshwater fishes (*Glossogobius giurus*, *Ophicephalus striatus*, *Therapon argenteus*) serve as first and second intermediate hosts, respectively. Water snakes (*Hurria rynchops*) and frogs (*Rana limnocharis*) may serve as the paratenic hosts locally.

Both *G. hispidum* and *G. doloresi* (Plate 8.17) have been recorded in pigs in the Philippines, but no case of human infection has been reported locally. However, cases of

human gnathostomiasis have been reported in China, Japan, and Thailand due to these two species. *Gnathostoma doloresi* is currently recognized as an important cause of clinical human gnathostomiasis in Japan.

Cases of human gnathostomiasis due to *G. hispidum* in Japan have been attributed to the consumption of the fish, *Misgurnus anguillicaudatus*. It is interesting to note that this fish now abounds in the rice terraces of Ifugao. The Ifugao call it *jojo*, which probably is derived from the Japanese name *dojo* for the fish. How the fish found its way to the Cordillera is not exactly known. It is postulated however that Japanese soldiers during World War II brought it as a protein supplement for their diet. This is a case of the introduction of a new suitable intermediate host for a parasite already existing in a country. The introduction increased the range of suitable intermediate host available locally and hence ensured further dissemination and continued survival of the parasite concerned.

In Japan, wild boars, salamanders, frogs, and snakes have been reported to harbor larvae of *G. doloresi*. In the Philippines, the larvae (Plate 8.18) of *G. doloresi* have been found in



Plate 8.17. *Gnathostoma doloresi* from pig
(Courtesy of Dr. Salcedo Eduardo)

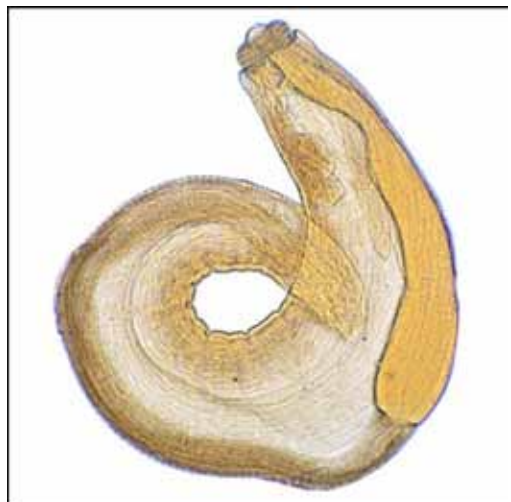


Plate 8.18. *Gnathostoma* larva from frog muscle
(Courtesy of Dr. Salcedo Eduardo)

frogs and *Ophicephalus striatus* (dalag) from Laguna Lake, suggesting that this fish serves as the intermediate host.

Human infection may result from consumption of improperly cooked infected fish or paratenic host, or through drinking water contaminated with infected copepods. The larva migrates to the subcutaneous tissues, central nervous system, and other tissues.

D. Zaprozoonoses

In this group, the causative agent of the infection develops from a non-infective to an infective stage in an environment containing organic matter including food, soil, or plant, or a reservoir before transmission to the vertebrate host (Figure 8.7).

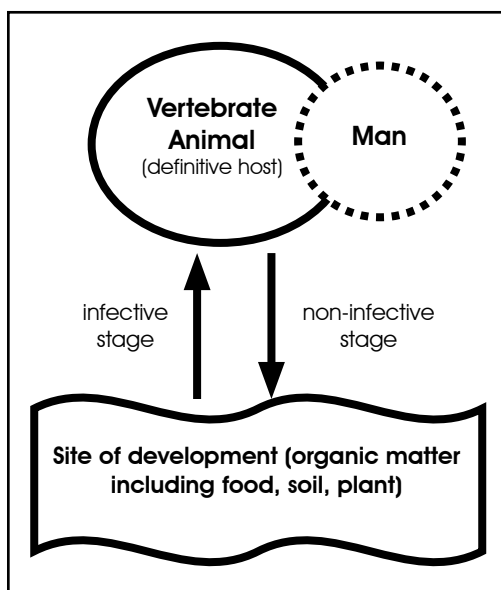


Figure 8.7. Zaprozoonoses

1. Larva migrans

This condition is caused by a wide range of nematode parasites of animals, the larva of which may invade skin (cutaneous larva migrans), viscera, and other organs (visceral larva migrans) of human, where they do not normally mature.

A number of animal hookworms (*Ancylostoma braziliense*, *A. caninum*, and *Bunostomum* spp.) and threadworms (*Strongyloides* spp.) are involved in cutaneous larva migrans. The first two species occur in dogs and cats, while the third occurs in ruminants. Threadworms are common intestinal parasites of mammals including humans, and many of the nonhuman species can cause larva migrans in humans. *A. braziliense* is the cause of creeping eruption. Human acquires the infection through contact with soil containing infective larvae. Normally larvae are restricted to and die in the skin but may also migrate to the lungs.

Toxocara canis (Plate 8.19), a common dog *Ascaris* is the main causative agent of visceral, ocular, and even covert larva migrans in human. Other ascarids like *T. cati* of cats and other felids, and *T. vitulorum* of cattle and water buffaloes may also be involved, but their role is limited due to the infrequency of human contact with their eggs. Puppies are infected with *T. canis* as early as the fetal stage or at birth due to transplacental and transmammary transmission from the infected bitch, and



Plate 8.19. *Toxocara canis* from dog
(Courtesy of Dr. Salcedo Eduardo)

are therefore the important source of eggs. Female *T. canis* are highly fecund and infected puppies may shed 100,000 eggs per gram of feces. Human becomes infected by ingestion of embryonated eggs (Plate 8.20) through contaminated food and water. Larvae migrate to all parts of the body including the eyes and brain. Other mammals and birds may serve as paratenic hosts. All the above species are present in the Philippines.

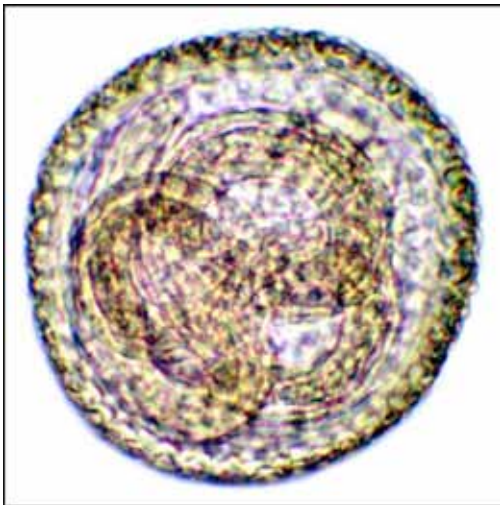


Plate 8.20. *Toxocara canis* embryonated egg (infective) (Courtesy of Dr. Salcedo Eduardo)

2. Mammomonogamiasis

Mammomonogamus laryngeus (Plate 8.21) is the causative agent of this condition and is a common parasite of ruminants (e.g., cattle, water buffaloes) in some parts of the world. In the Philippines, 23% of slaughtered cattle in Cagayan de Oro City were found infected with this parasite. *M. laryngeus* has been recovered from the trachea of water buffaloes slaughtered in Bayog, Los Baños, Laguna (unpublished).

Human infection results from ingestion of embryonated ova or infective larvae through contaminated food and water, or accidental ingestion of transport hosts such as earthworms, snails, or arthropods. To date, about 100 cases



Plate 8.21. *Mammomonogamus laryngeus* in copula from water buffalo (Courtesy of Dr. Salcedo Eduardo)

of human infections have been recorded from the Caribbean Islands, Brazil, Korea, Thailand, and the Philippines.

The Role of Eating Habits and Practices in the Transmission of Parasitic Zoonoses

The important role played by food habits and practices in the epidemiology of a number of these zoonoses is evident from the above summary.

Food dishes prepared as raw or partly cooked are relished in some areas in the Philippines. *Kilawen* is a term given to any preparation of raw meat, fish, snail, shrimp, or crab, usually with salt, vinegar, and spices. This kind of preparation is considered a delicacy in some parts of the country. Thus, human cases of echinostomiasis, artyfechinostomosis, and intestinal capillariasis have been described in such areas. In Isabela, where human cases of artyfechinostomosis have been reported, the snail second intermediate host is eaten raw or partly fermented. It is prepared by shaking the snail with salt to remove mucus secretion, then salt, ginger, onion, vinegar, pepper, and other spices are added and eaten or left overnight to ferment before being consumed. This is in contrast with another place, San Pablo, Laguna in Southern Luzon, where the same snail has been found to have even higher percentage of infection than in Isabela, but no case of human infection has occurred since this snail is not eaten by the local population. Instead, it is

detested due to its slimy texture. Even the local term, *susong linta*, meaning “leech-like” snail, sounds unpleasant to the ear.

Kilawen is also popular among folks in Leyte. Pig liver is cut into thin slices, soaked in vinegar with salt and condiments and eaten raw. Pig meat, partly cooked and prepared as above, is also eaten. *Cysticercus* (larva) of *Taenia solium* and *T. saginata asiatica* are found in the muscle and liver respectively of pigs, which serve as intermediate hosts. Human infection occurs through consumption of raw or partly cooked infected organs.

Pila conica (*kuhol*, *bisukol*) and *Sundathelpusa philippina* (*talangka*), the second intermediate hosts of *E ilocanum* and *P. westermani filipinus*, respectively, are eaten practically all over the country. However, echinostomiasis and paragonimiasis are prevalent or endemic only in certain areas. In Northern Luzon, echinostomiasis has the highest prevalence as the snail host is eaten sometimes raw or partly cooked in this area. Similarly, paragonimiasis is endemic in areas where inhabitants are known to consume the crab host raw. A preparation with fresh crab juice locally known as *kinagang* is considered a local delicacy. Males in these areas were observed to eat raw crabs during drinking sessions with the local wine (*bas*), especially during festivities. The same is true for intestinal capillariasis in Northern Luzon. The fish host, *Hypseleotris bipartite*, is especially desired when gravid (filled with eggs), and the entire fish is eaten raw. Another fish host, *Ambassis miops*, in the raw form, is bitten at the belly by some to suck out the juice. Residents of endemic areas in Northeastern Mindanao were also noted to consume fish raw.

Simply giving up the habit of eating raw food of animal origin may prevent human infection with a number of these zoonoses. However, as the saying goes, old habits may not easily be given up. Furthermore, some people in these areas, though properly informed about this transmission, still value their food habits.

Some maintain that cooking not only destroys the flavor they relish, but also the nutritive value of the food. Nevertheless, with a more aggressive health education campaign, together with programs directed to the improvement of the living condition of the inhabitants in these areas, preventive measures against many of these zoonotic diseases can be achieved successfully.

Zoonotic Parasites as Indicators of Fecal Pollution of the Environment

Many of the protozoa and helminth agents causing zoonoses described in this section are associated with fecal pollution of the environment, whether land or aquatic. Many protozoan and helminth parasites shed cysts (*Balantidium coli*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia duodenalis*, *Toxoplasma gondii*, *Sarcocystis* spp.) and eggs/larvae (*Toxocara canis*, *Ancylostoma* spp., *Strongyloides* spp.), respectively, that are disseminated to the environment through the feces. Furthermore, many helminth parasites require intermediate hosts to complete their cycle (cyclozoonoses and metazoonoses). The eggs/larvae of the parasites are passed out with the feces of the definitive host to the environment before they develop and gain access to the intermediate host.

Echinostoma spp., *Artyfechinostomum malayanum*, *Fasciola* spp., heterophyids, *Carneophallus brevicaeca*, *Plagiorchis* spp., *Schistosoma japonicum*, *Paragonimus westermani*, and *Capillaria philippinensis* reach their intermediate hosts through fecal contamination of the water environment. *Sarcocystis* spp., *Eurytrema* spp., *Taenia* spp., and *Macracanthorhynchus hirudinaceus* reach their intermediate hosts through fecal contamination of the pasture or direct access of the intermediate host (pig and cattle) to fecal matter of infected definitive host (humans) in case of human taeniasis. Proper disposal of fecal material, whether of humans or animals, therefore, is everyone's concern.

The presence of reservoir hosts in combination with the presence of suitable invertebrate intermediate hosts also maintains the infection in a particular area. Field rats are maintaining the cycle of zoonotic parasites such as *Echinostoma* spp., *A. malayanum*, *Schistosoma japonicum*, *Plagiorchis* spp., and *Paragonimus westermani*. Because of the intermediate hosts, the potential risk of human infection is ever present. The snail, *Bullastra cummingiana*, had high prevalence of infection with *A. malayanum* in Sampaloc Lake in San Pablo City. It should be noted that piggeries are concentrated around Sampaloc Lake, and their excreta pollute the lake. Pigs, apart from rats, are known as a definitive host of the parasite and are maintaining the cycle in that area. While there are no human cases of infection as yet, the presence of the parasite in the area still poses a threat to human health.

Economic Losses Resulting from Parasitic Zoonoses

The Philippines has a fast growing human population and currently has already reached 94 million. It is even projected that at a growth rate of 2% annually, the population may reach 113 million by the year 2020. However, food animal production has not increased to keep pace with the demand of the increasing human population. Recent statistics revealed a slow growth for food animal production. A large proportion of the human population live in the rural areas, and a much larger proportion of the food animal population are raised in the backyard. This food animal population remains large compared to the number raised in large commercial farms. This ecological profile of human and animal population distribution makes a large proportion of both populations at risk of infection.

Although it is difficult to assess exactly economic losses from zoonoses, it is evident in the Philippine setting that these diseases are prevalent in rural areas where the population

depends on agriculture, fishery, and forestry for livelihood. One-third of all goods and services produced by the economy is accounted for by the agricultural/rural sector, which also employs half of the country's workers, and earns 36% of the country's export income. An unhealthy working population can only mean low or reduced productivity, while infected animals mean unwholesome meat such as in cases of fascioliasis and cysticercosis. This, in turn, can only lead to further reduction of supplies due to carcass condemnation of what is already an insufficient meat supply. In a country like the Philippines where poverty is widespread in rural areas, these diseases can only worsen what is already a bad situation.

Medical-Veterinary Cooperation in the Control of Parasitic Zoonoses

Animals, both invertebrates and vertebrates, domesticated and wild, are hosts to a number of parasitic zoonoses as already shown in the above discussions. Their role as definitive, intermediate, and reservoir hosts make them essential in maintaining the zoonotic agents in nature. As paratenic or transport hosts, they prolong the availability of the agent as potential sources of human infection, as well as increase the potential of disease dissemination.

Studies to better understand the processes involved in the maintenance, transmission, and epidemiology of these diseases should involve the participation of those concerned, especially physicians, veterinarians, and public health workers. In most countries including the Philippines, parasitic zoonoses are the most underdiagnosed diseases in human. Some human infections with these diseases may have passed unnoticed or may have been misdiagnosed, as some are difficult to detect or many simply are not aware of them.

The control of zoonoses involves: control in animals, the veterinarian's concern; prevention and treatment in humans, the physician's responsibility; and the control

of vehicles of transmission, the concern of both. For the management and control of zoonoses to be effective and successful, joint medical and veterinary efforts are necessary. Medical-veterinary cooperation has been more prominent in parasitology than in any other area of medicine. It should continue to flourish and should be fully supported.

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Immunocompromised Hosts and Parasitic Infections

Edsel Maurice T. Salvaña, Evalyn A. Roxas, Mary Ann D. Peñamora

Parasites are uniquely adapted to survive and thrive in the human host despite the presence of a hostile immune system. In order to defeat this protection, parasites deploy a number of strategies to either evade or overwhelm natural host defenses. Immunocompromised individuals are at a distinct disadvantage in seeking to detect and control parasitic infections. Moreover, they become susceptible to otherwise non-pathogenic organisms which then cause opportunistic infections. In this chapter, we review some of the more common opportunistic parasitic infections and describe strategies to prevent and treat these diseases.

Etiology and Pathogenesis

The end goal of a parasite is to reproduce and perpetuate the species. Therefore, survival strategies dictate that the host survives long enough for the parasite to propagate and spread. There is evidence that the most successful parasites cause little or no disease as a consequence of millions of years of co-evolution with its host. For instance, we know that the least pathogenic of the malaria species, *Plasmodium malariae*, is the oldest human parasite among the group, and that the most pathogenic, *P. falciparum*, is the most recent to cross over to humans. Hosts, specifically human hosts, should be thought of as not just one organism but as a tightly balanced environment with endogenous flora with unique ecological niches. This milieu is a product of the interactions of the host immune system and the microbial flora, and perturbations in either can upset the health of the organism. Various conditions which compromise the immune system will affect the balance between the pathogen and host, and allow organisms to replicate at higher

than normal levels, producing more severe disease. This opportunism can be exemplified as infection by parasitic organisms which are rarely pathogenic (e.g., *Acanthamoeba*, microsporidia) or those which cause increased disease severity or duration (e.g., *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, *Toxoplasma*, *Strongyloides*).

Multiple processes can predispose to infection by compromising the anatomical and physical barriers of the host. Defects in the inflammatory pathways and immune functions may allow infections by opportunistic pathogens. Some of these defects may be related to a congenital disorder or abnormal development, underlying acquired disease, drug therapy, malignancy or irradiation. Specific defects in immune components lead to susceptibility to specific subsets of pathogens, such that the pattern of opportunistic infections is a clue to the underlying defect. In addition, once established, some pathogens such as protozoans can further exacerbate or produce other abnormalities in immunologic function such as in the case of *T. gondii*, *Leishmania*, or *Plasmodium* spp. The consequences of protozoan or helminthic diseases which cause malnutrition may alter immune function. These alterations are not necessarily associated with clinical susceptibility to opportunistic pathogens per se but may contribute to a less than ideal host response. Finally, one must consider that some immune defects may be mixed, with both humoral and cell-mediated components; and that because there is some overlap between components, deficiency in one component may still adversely impact another component that otherwise has all its elements in working order.

A. Leukocyte Deficiencies

Predilection to parasitic infection in leukocyte disorders depends on the type of leukocytes which are numerically or functionally affected, and on how prolonged the dysfunction is. The rate of decline and duration are also important parameters that influence clinical outcome. Neutropenia ($<1,000$ neutrophils/ mm^3) is the most commonly encountered defect in inflammatory host defense mechanisms. Susceptibility to bacterial and fungal infections, but not usually to protozoan, viral or helminthic disease, increases dramatically when the peripheral neutrophil count falls below 500 cells/ mm^3 and increases more markedly when the count falls below 100 cells/ mm^3 . Lymphopenia in adults is defined as having less than 1,000 lymphocytes/ mm^3 . Its clinical consequences depend on which lymphocyte subsets are affected. Regardless of the total lymphocyte count, severe infections of various types may occur if profound deficiencies of either B-lymphocytes or T-lymphocytes are present. Substantial reductions in helper T-lymphocytes have important consequences in terms of susceptibility to protozoan (*Toxoplasma*) and helminthic (*Strongyloides*) infections. The most common causes of lymphopenia are hematologic malignancies, corticosteroid therapy, anti-lymphocyte globulins, cytotoxic drugs, and infections with certain viruses such as cytomegalovirus and the human immunodeficiency virus (HIV), the etiologic agent of acquired immune deficiency syndrome (AIDS).

B. Humoral Deficiencies

Immunoglobulin deficiencies particularly those that affect IgG and IgA production can cause a marked increase in susceptibility to infections caused by *Plasmodium* spp., *Babesia*, and *Giardia*. Patients with significant reduction in IgG (usually <200 to 300 mg/dL) characteristically have recurrent infections

due to encapsulated bacteria and protozoans. Development of immunity to invasive pathogens such as *Entamoeba histolytica* can be impaired, and amebic disease may progress rapidly. The spleen is a major site for T-cell independent immune responses and large numbers of B-lymphocytes, monocytes, and macrophages reside. It has a prominent role in the phagocytosis of circulating opsonized organisms. In several reported cases, malaria appears to be more severe after splenectomy, while babesiosis, as a clinical disease seems to occur with unusually high frequency. The clinical importance of splenectomy for other protozoans and helminths is less clear. Complement deficiencies can affect the clearance of organisms, but whether these predispose towards specific parasitic infections is less clear. Treatment with omalizumab, a monoclonal antibody against IgE for asthma has led to some concern of predisposition towards helminthic infection, but at least one randomized control trial has shown no additional significant risk.

Understanding the specific immune deficiency in an immunocompromised patient is particularly important with regard to protozoans and helminthic infections since appropriate diagnostic tests for these diseases may not be routinely requested or may not be available; and empiric regimens do not usually include protozoan or helminthic coverage. Moreover, matching these defects with common, endemic causative organisms in a given locale is essential.

Protozoans and Helminths of Special Importance

Protozoans and helminths that are of special concern to immunocompromised individuals are listed and summarized in Table 8.3. Multiple parasitic infections are more likely to occur in immunocompromised individuals as well. This is especially true in AIDS. HIV and AIDS have resulted in a sharp increase in the number of

Table 8.3. Protozoans and helminthic organisms of special importance to immunocompromised patients

Protozoans	Helminths
<i>Toxoplasma gondii</i>	<i>Strongyloides stercoralis</i>
<i>Cryptosporidium</i> spp.	<i>Filaria</i> spp.
<i>Cystoisospora belli</i>	
Microsporidia	
<i>Cyclospora</i> spp.	
<i>Giardia duodenalis</i>	
<i>Blastocystis hominis</i>	
<i>Entamoeba histolytica</i>	
<i>Babesia</i> spp.	
<i>Leishmania</i> spp.	
<i>Trypanosoma</i> spp.	
<i>Plasmodium</i> spp.	

cases of life-threatening opportunistic infections due to bacteria, fungi, viruses, and parasites.

A. HIV and Parasitic Infection

HIV depletes the helper (CD4+) subset of T-lymphocytes with drastic consequences on cell-mediated immunity. Unmodulated inflammation and immune activation increase susceptibility to a host of illnesses and malignancies, and may allow previously latent infections to become active. These latent infections include tuberculosis, herpes viruses, *Leishmania*, and *Toxoplasma*. Low pathogenicity organisms in immunocompetent hosts such as *Pneumocystis jirovecii* and intestinal sporozoans may develop into life-threatening infections. Finally, pathogens which may be mild or less severe such as *Babesia* and *Plasmodium* may become more virulent as a result of the permissive environment.

The pattern and types of HIV-related opportunistic infections throughout the world is affected by endemic infections, general health, nutrition, and access to health care and medical services. The dramatic frequency of parasitic infections in AIDS presents an important lesson about the interrelationship

between protozoans or helminths and cellular immunity. Uncontrolled enteric parasite infection contributes to increasing malnutrition from malabsorption and direct damage, which in turn further taxes the immune system. Metabolic derangements from HIV infection itself further contribute detrimental effects to the host.

A study by Chaisson et al. on the impact of opportunistic diseases in the United States among a cohort of 2,081 patients with HIV infection between 1989 and 1995 revealed a total of 1,499 (49%) opportunistic diseases during follow-ups. The predominance of enteric protozoans, especially *Cryptosporidium*, as causes of chronic diarrhea has been reported to occur in 30 to 60% of AIDS patients in Haiti, Africa, and other developing countries. Infections with microsporidia, *Cystoisospora*, *Giardia*, and other rarer organisms have also been reported. Enteric protozoans are among the important etiologic agents of diarrhea in AIDS in Thailand. Of 288 patients screened over a 10 month period in 1999 to 2000, 55 (19.2%) had *Cryptosporidium* spp., 13 (4.5%) had *Cystoisospora* oocysts, 11 (3.8%) had *G. lamblia*, 3 (0.9%) had *Entamoeba histolytica*, and 1 (0.3%) had *Iodamoeba butschlii* infection. The prevalence of microsporidia was 11% in this study. Studies in Dakar, Senegal by Gassama, et al. among HIV-infected and non-infected patients with diarrhea revealed *Microsporidium* (9.4%), *Cryptosporidium* sp. (8.2%), *E. histolytica* (5.1%), and *Cystoisospora belli* (4.4%) to be the more frequent parasites seen among the immunocompromised individuals and were often identified in patients with low CD4+ count. *Blastocystis hominis* was identified only among HIV-infected individuals. Additionally, high levels of asymptomatic carriage of *A. lumbricoides* and *T. trichiura* were observed.

A follow-up study on the progression of HIV infection performed by Manaloto et al., in a cohort of 54 HIV-infected Filipino commercial sex workers from May 1985 to

July 1992 revealed *Mycobacterium tuberculosis* and *Pneumocystis carinii* pneumonia as the initial indicators of immunodeficiency following a CD4⁺ cell count of <200 cells/mm. Cryptosporidiosis and brain toxoplasmosis were also seen in two patients. Among the 145 patients with HIV infection seen at the Research Institute for Tropical Medicine (RITM) from 1985 to 1996, cryptosporidiosis was diagnosed in 31% of cases. *G. lamblia* was detected in 13%, *Ascaris lumbricoides* in 11%, *E. histolytica* in 9%, *E. nana* in 7%. *B. hominis*, *H. nana*, and *T. trichiura* each in 2% of the cases. Follow-up of 103 symptomatic cases through 1998 did not reveal significant differences in the prevalence of the parasitic infections seen previously. Additionally, 2% of the cases revealed CNS toxoplasmosis and 3% had *B. hominis*.

B. *Toxoplasma gondii*

Toxoplasma gondii is a sporozoan in parasite that infects up to a third of the world's population. It infects all orders of mammals and some birds. The domestic cat is a definitive host and produces infective oocysts. Handling of cat feces is a strong risk factor for contracting primary disease. Ingestion of food or water contaminated with oocysts, and eating of undercooked meat is the usual means of infection. *Toxoplasma gondii* can be passed transplacentally to the fetus when a pregnant woman has a primary infection, leading to fetal infection leading to severe congenital anomalies. The prevalence of *Toxoplasma* antibodies varies considerably among different populations and ranges from 3 to 70% in the United States to as high as 80% in Western Europe.

Toxoplasma is an intracellular parasite capable of invading and replicating within nucleated cells. Ingested oocysts enter host cells either by rupturing the membrane or by invaginating them. After multiplication by repeated endodyogeny, the macrophage finally ruptures, liberating the replicating stage (tachyzoites) of the parasite and giving rise to the

formation of pseudocysts and cysts that contain a more slowly replicating stage (bradyzoites). The cysts are distributed throughout the body. In the central nervous system, they appear to persist in latent form for the entire lifespan of the host, provoking little if any inflammatory response. These dormant organisms can be reactivated in immunosuppressed persons. In recent years, the importance of toxoplasmosis in immunocompromised host has been increasingly recognized. Patients with a variety of neoplastic diseases, including Hodgkin's lymphoma, as well as patients receiving immunosuppressive therapy are at risk of reactivation of this infection. The incidence of toxoplasmosis has raised dramatically with the increasing population of AIDS patients. *T. gondii* is now the leading cause of space-occupying cranial lesions in persons with AIDS.

Infection of immunologically normal persons with *Toxoplasma* usually results in a persistent but asymptomatic infection in 80 to 90% of patients. Primary disease is also usually subclinical but in some patients may present as a mononucleosis-like syndrome with cervical lymphadenopathy and rarely with ocular manifestations.

Toxoplasmosis in AIDS patients usually develops at CD4 counts of less than 100 cells/mm³. While virtually any organ may be involved, the most common manifestations are in the central nervous system and may involve the eyes. Virtually all toxoplasmosis in AIDS patients is reactivation, and so only *Toxoplasma* IgG positive patients are considered at risk. Other underlying conditions that may give rise to reactivation of toxoplasmosis include various malignancies (such as Hodgkin's disease, non-Hodgkin's lymphomas, leukemias, and solid tumor collagen vascular disease, organ transplantation, and prolonged steroid use). More than 50% of these patients show altered mental status, motor impairment, seizures, abnormal reflexes, and other neurologic sequelae. The most common presenting

symptom is still seizure, followed by focal neurologic deficits including ocular symptoms.

Diagnosis of acute disease is through detection of IgM antibodies or a four-fold rise in antibody titer. The presence of high titers ($>1:1024$) by the Sabin-Feldman dye test, direct agglutination tests, or conventional indirect immunofluorescent antibody (IFA) technique is suggestive of acute infection. However, high antibody titers may persist for years after infection. Therefore, in patients with stable high titer and detection of IgM antibody by the IgM-IFA double sandwich IgM enzyme immunoassay (EIA), or immunoblot tests may be useful. Other assays include complement-fixation test and conventional IgA-EIA. A negative IgM test essentially excludes recent infection, but a positive IgM test is difficult to interpret because *Toxoplasma*-specific IgM antibodies may be detected by EIA for as long as 18 months after acute acquired infection.

Detection of IgG antibodies indicates prior infection and the possible presence of tissue cysts. In the immunocompromised hosts, interpretation of serological test is dependent on understanding of the degree of underlying immunosuppression, the serological status of the patient prior to the development of symptoms indicating acute *Toxoplasma* infection, and knowledge of the pathogenesis of *Toxoplasma* infection in the risk group to which the patient belongs (e.g., transplant recipients). Serologic tests may reveal changes in antibody titers without necessarily being indicative of active infection. Therefore, serological rises in antibody titers in immunocompromised patient cannot be used as the sole diagnostic criterion of active infection with *Toxoplasma*, especially if the clinical manifestations are non-specific. In contrast to rises in antibody titers in some immunocompromised patients without any definite signs or symptoms of active toxoplasmic infection, other immunocompromised patients with fulminant toxoplasmosis may have low or negative dye test or IFA titers and show no

rise in antibody titer with serial specimens. Since the interpretation of serological tests for toxoplasmosis is not uniform, it must be correlated with other diagnostic techniques including radiographic and other laboratory abnormalities as well as the clinical situation.

The detection of *Toxoplasma* antigen in serum or other body fluids (e.g., CSF, ocular fluid, urine) may be particularly important in immunocompromised patients in whom active disease is not always associated with rises in antibody titers. The gold standard for diagnosis remains demonstration of the organism in tissue.

Toxoplasma gondii has been identified in biopsy specimens of the bone marrow, myocardium, skeletal muscle, lung, and brain using both hematoxylin and eosin (H&E) stain and immunospecific stains for *Toxoplasma*. Biopsy samples can also be inoculated into mice or sensitive cell lines to isolate the organism. However, because many individuals have been exposed to *Toxoplasma* and may have cysts within tissues, recovery of the organism from cell culture or animal inoculation maybe misleading. Recently, the use of molecular technology techniques (such as PCR, DNA hybridization using ABGTg7 probe) have been found to be sensitive, specific, and rapid methods for the detection of *T. gondii* DNA in amniotic fluid, blood, BAL fluid, tissue samples, and CSF. These are currently research tools and are considered ancillary diagnostics especially when only very small amount of specimen is available, when the condition is dubious, when the result is required urgently or if serological tests are inconclusive. Radiologic examinations such as computerized axial tomography (CAT) scan and nuclear magnetic resonance imaging (MRI) have been found to be extremely useful in the demonstration of abnormalities associated with TE in patients with no underlying immunosuppression as well as in immunocompromised hosts. In AIDS patients, the most significant differential

diagnosis is central nervous lymphoma, and differentiation can be quite difficult. If the mass is small and there are no life-threatening complications, empiric treatment followed by serial MRI's to document improvement can be done. However, in cases where diagnosis is urgent and delay can lead to serious clinical consequences, brain biopsy must be pursued.

Treatment for *Toxoplasma* infections is indicated for patients who develop acute infection during pregnancy, and for immunocompromised patients with evidence of reactivation disease. The combination of pyrimethamine and sulfadiazine is the most effective regimen. Empiric therapy should be instituted in seropositive immunocompromised patients who present with compatible neurologic symptoms and characteristic imaging. Asymptomatic patients may become symptomatic and symptomatic patients may briefly worsen when initiating antiretroviral therapy for HIV due to immune reconstitution.

Trimethoprim-sulfamethoxazole (TMP-SMZ) when used as prophylaxis for *Pneumocystis jiroveci* is effective prophylaxis for toxoplasmosis. If TMP-SMZ cannot be tolerated, there are alternative prophylactic regimens which include clindamycin and dapsone plus pyrimethamine. Atovaquone with or without pyrimethamine may also be considered. Patients with a history of central nervous system toxoplasmosis should be administered suppressive therapy with drugs active against *Toxoplasma* to prevent relapse, until the CD4 count is above 100 for over a year, or the initial immunosuppressing condition has resolved.

Immunocompromised patients should be tested for IgG antibody to *Toxoplasma* to detect latent infection and offered prophylaxis as appropriate. Seronegative patients should be counseled about the various sources of toxoplasmic infections and advised appropriate methods of preventing exposure especially. Because infection is usually transmitted by ingestion of undercooked meat with viable

cysts or by food contaminated with oocysts, susceptible patients should not eat raw or undercooked meat and should thoroughly wash, peel or blanch fresh produce. Careful hand washing after handling potentially contaminated material including cat litter, raw meat, and fresh produce is essential. The presence of a cat at home is a risk for infection, and steps should be taken to minimize contact between the cat and the patient, and if unavoidable, the patient should follow strict hand washing.

C. *Cryptosporidium*

Cryptosporidium was initially described in mice in 1907, but it was not until 1976 that it was first reported in humans. The advent of the AIDS epidemic substantially increased the number of cases. *Cryptosporidium* is an intestinal spore forming protozoa which mainly causes diarrheal illness. In otherwise healthy individuals, *Cryptosporidium* sp. typically causes watery or mucoid diarrhea with abdominal pain lasting for several days or occasionally weeks that is self-limited even without treatment. *Cryptosporidium* causes far more serious disease in immunocompromised individuals, with no effective treatment for those with AIDS.

The most commonly identified species considered pathogenic for man is *C. parvum*. Two genotypes of *C. parvum* are responsible for most human infections. These include the human anthroponotic genotype 1 found almost exclusively in humans and the bovine or zoonotic genotype 2 found in both ruminants and human. However, studies revealing molecular diversity among human *Cryptosporidium* isolates suggest that multiple subgenotypes or more than one species may be implicated in human disease.

Experimental-infection studies with mice and calves show that immunity is dependent on the number of CD4 T-cells generating gamma interferon. No difference was found between cryptosporidiosis in normal and B-cell-depleted neonatal mice, suggesting that

antibody production may play a less important role in recovery from infection. Interleukin-12 also plays a role by inducing gamma interferon production.

All species of *Cryptosporidium* that have been studied are obligate intracellular parasites, however, unlike other coccidians, their developmental stages do not occur deep within the host cells but are confined to an extracytoplasmic location. Each stage is within a parasitophorous vacuole within the microvillous region of the mucosal epithelium of several organs including the respiratory tract and the biliary tract, but most commonly that of the gastrointestinal tract. *Cryptosporidium* differs from other coccidians in its ability to undergo complete development within a single host. The sporozoites, after being released from the host cell, can penetrate the microvillous region of other cells and reinitiate the life cycle. Oocysts excreted in stool are immediately infective to the same host and to others. This auto-infective capability contributes to the refractory nature of cryptosporidial infection in patients with impaired immunity.

Cryptosporidium is ubiquitous around the world, with the highest prevalence observed in less developed countries. It is transmitted via contaminated food or water. *Cryptosporidium* contamination of surface water is quite common. The number of ingested *Cryptosporidium* oocysts required to cause illness is quite low, with median human infective dose of 132 oocysts.

Cryptosporidiosis is the most common cause of waterborne disease in the United Kingdom. In the United States, the Milwaukee cryptosporidiosis outbreak in 1993 was the largest outbreak of waterborne disease ever reported in the United States due to Lake Michigan water contaminated with *Cryptosporidium* oocysts. An estimated 403,000 residents and visitors of Milwaukee experienced watery diarrhea and 54 cryptosporidiosis-associated deaths occurred during the two-year

post outbreak period compared with four deaths overall in the two years before the outbreak. This represented a more than a 13-fold increase in cryptosporidiosis-associated mortality.

Zoonotic and person-to-person transmission may occur through direct or indirect contact with stool material in the environment, day-care centers, and the hospital setting. Direct transmission may occur sexually during oral-anal contact. Indirect contact may occur through exposure to positive specimens in the laboratory setting or from contaminated surfaces or food or water. Studies have shown that calves and other animals, including kittens, rodents, puppies, and birds may serve as potential sources of human infections. *Cryptosporidium* oocysts, are resistant to most disinfectants, and are difficult to filter due to their small size, thus enabling them to persist and spread in the environment.

Cryptosporidiosis is a substantial threat to HIV infected individuals, who have a lifetime risk of infection of around 10%. The most common clinical feature of cryptosporidiosis is diarrhea. Among adult HIV patients, cryptosporidiosis is the reported cause of diarrhea in 15 to 40%.

C. parvum infections are not always confined to the gastrointestinal tract; additional symptoms (respiratory problems, cholecystitis, hepatitis, and pancreatitis) have been associated with extraintestinal infections. Chronic cough, dyspnea, and fever have been reported to be the major symptoms in pulmonary cryptosporidiosis, with diarrhea only as an associated symptom.

Diagnostic techniques include stool examination, histologic examination of intestinal biopsy, and examination of duodenal aspirates. *Cryptosporidium* oocysts in the stool range from 4 to 6 μm in diameter and can be very difficult to identify. Stools and other body fluid specimens (e.g., sputum) should be submitted as fresh material or in 5 or 10% formalin, sodium acetate-acetic acid-formalin (SAF),

or polyvinyl alcohol (PVA) with zinc sulfate-based Schaudinn's fixative. Fixed specimens are recommended because of potential biohazard considerations. Some techniques have included sugar flotation, formalin sedimentation, Giemsa stain, trichrome, periodic acid-Schiff (PAS), silver methenamine, acridine orange, auramine-rhodamine, iodine, modified acid-fast, Kinyoun and Ziehl-Neelsen acid-fast, immunofluorescence assay and immunoassay methods. Immunoassay procedures for the direct detection of *Cryptosporidium* antigen or oocysts in fecal specimens have proven to be much more sensitive than the routine acid-fast stains. Enzyme immunoassays, solid-phase immunochromatographic assays, and immunofluorescence assays, which use monoclonal antibodies against the oocyst wall, are currently available. A flow-cytometric method for the quantitation of *Cryptosporidium* oocysts in stool specimens have been developed as an alternative method, however, the approach appears to be somewhat impractical. PCR technology also offers alternatives to conventional diagnosis and allows the differentiation of *Cryptosporidium* genotypes. Antibody assays using crude extracts of disrupted oocysts or recombinant antigens of *Cryptosporidium* in an ELISA format and specific *Cryptosporidium* antigens by immunoblot method have been used for the diagnosis and monitoring of *Cryptosporidium* infections.

Although many therapeutic regimens have been tried, there is no completely satisfactory therapy for cryptosporidiosis in humans. A recent meta-analysis of trials of antiparasitic drugs in cryptosporidiosis noted significant improvement of non-AIDS patients with nitazoxanide, but no clear evidence of efficacy for other antiparasitic drugs in cryptosporidiosis or for nitazoxanide in AIDS patients. Drugs that have been tried in different regimens include paromomycin plus azithromycin, clarithromycin, and hyperimmune bovine colostrums. In a randomized controlled trial of

Cryptosporidium-infected HIV patients in India, the efficacy of short-term azithromycin in the management of cryptosporidiosis was studied. Short-term azithromycin (500 mg once daily for 5 days) treatment for cryptosporidial diarrhea in AIDS patients was associated with good clinical improvement but parasitological benefit was doubtful. All 13 patients, who had symptoms of cryptosporidiosis, symptomatically improved with 5 days of treatment with azithromycin and became asymptomatic after 7 days of antibiotic, but the stool sample remained positive for *Cryptosporidium* even after 7 days of therapy. After 14 days of treatment with azithromycin in 13 patients, stool samples from five patients were free of cryptosporidial oocyst. The drug was well tolerated in all the patients. This small study suggests that short-term azithromycin can be used as a safe and effective treatment for symptomatic cryptosporidiosis but is not effective in eradicating cryptosporidial infection. Supportive measures are important in the management of cryptosporidial diarrhea. Nutritional supplements and anti-diarrheal agents may be necessary for symptomatic treatment of severe disease. In the absence of effective therapy, prevention of infection is paramount. Immunocompromised patients, especially HIV-infected persons, should be educated and counseled about *Cryptosporidium* acquisition and transmission. They should be advised to avoid contact with feces and to wash their hands after handling pets or contact with soil. Patients should avoid sexual practices that might result in oral exposure to feces (e.g. oral-anal contact). Cryptosporidiosis may be acquired by drinking contaminated water or contact with contaminated water during recreational activities. Water from suspect sources should be boiled or filtered, and at risk patients should refrain from swimming in fresh water. Since patients with cryptosporidiosis eliminate large amounts of oocysts in their feces, they can easily contaminate the environment and persons in contact with them. Because

of this, some experts recommend that HIV-infected persons or other immunocompromised patients should not share a room with a patient with known cryptosporidiosis.

D. *Cystoisospora belli*

Cystoisospora belli is another sporozoan that causes diarrhea in immunocompromised hosts. These organisms can infect both adult and children, and intestinal involvement and symptoms are generally transient unless the patient is immunocompromised. *C. belli* is thought to be the only species of *Cystoisospora* that infects humans, and no other reservoir hosts are recognized for this infection. Transmission is through ingestion of food or water contaminated with mature, sporulated oocysts. Sexual transmission by direct oral-anal contact has been postulated. The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist.

Schizogonic and sporogonic stages have been found in the epithelial cells of the distal duodenum and proximal jejunum of the intestines. Eventually, oocysts are passed in the stool. Oocysts continue to mature within 48 hours after stool evacuation and are then infectious. Chronic infections develop in some patients and oocysts can be shed for several months to years.

Patients who are immunocompromised, particularly those with AIDS, often present with profuse diarrhea associated with weakness, anorexia, and weight loss. Bowel movements are watery, soft, foamy, and offensive smelling, suggestive of a malabsorption process.

Aside from AIDS patients, *C. belli* has been reported to cause opportunistic diarrhea in patients with Hodgkin's disease, non-Hodgkin's, human T-cell leukemia, and acute lymphoblastic leukemia. A case report in Iran described a patient with mediastinal thymoma with an eight-month history of recurrent diarrhea. On direct fecal smear

and duodenal and colonic mucosal biopsies, numerous *Cystoisospora* oocysts were detected. Extraintestinal infections, including biliary tract, respiratory tract, lymphatic channel, and spleen involvement, have been reported. Relapse tends to be common and may be associated with extraintestinal stages. Charcot-Leyden crystals derived from eosinophils have also been found in stools of patients with *C. belli* infection.

Diagnosis is made by examination of a fecal specimen for oocysts. Wet mount examination either by direct smear or concentrated material allows the demonstration of very pale and transparent oocysts. They appear long and oval measuring 20 to 33 μm by 10 to 19 μm in size. One or, less commonly, two immature sporonts may be present as well. Similar to other coccidians, acid-fast and auramine-rhodamine staining can be used to demonstrate organisms in stool.

Effective treatment is with TMP-SMZ, pyrimethamine-sulfadiazine, primaquine phosphate-nitrofurantoin, or primaquine chloroquine phosphate. TMP-SMZ is the drug of choice. Therapy must be continued indefinitely for immunosuppressed or immunocompromised patients with recurrent or persistent cystoisosporiasis. Since transmission is via infective oocysts, meticulous hygiene and sanitation are essential for preventing spread of the disease.

E. *Cyclospora cayetanensis*

Cyclospora cayetanensis is an acid-fast variable enteric coccidian that can infect travelers in developing countries as well as immunosuppressed hosts including AIDS patients. Spherical unsporulated oocysts, 8 to 10 μm in size (twice the size of *Cryptosporidium*) or ovoid sporocysts, 4 by 6.3 μm in size, are passed in the stools, and sporulation occurs within approximately 7 to 13 days. Complete sporulation produces two sporocysts that rupture to reveal two crescent-shaped sporozoites measuring 1.2 by 9.0 μm . The transmission of

Cyclospora is thought to be fecal-oral, although direct person to person transmission has not been documented and may not occur since sporulation takes a number of days. Outbreaks linked to contaminated water and various types of fresh produce such as raspberries, basil, and lettuce have been reported.

Cyclospora infection causes disease manifestations typical of a small bowel pathogen, including upper gastrointestinal symptoms, malabsorption, weight loss, and moderate to marked erythema of the distal duodenum. Two to 11 days following exposure, malaise and low grade fever develops and watery diarrhea ensues. Associated symptoms include extreme fatigue, anorexia, myalgia, vomiting, and weight loss, with spontaneous remission of diarrhea in 3 to 4 days followed by frequent relapses lasting from 4 to 7 weeks. AIDS patients may take longer to resolve (up to 12 weeks) and may develop chronic diarrhea if treatment is not initiated. Biliary disease is a known complication. The clinical presentation of patients infected with this organism is similar to that of patients infected with *Cryptosporidium*. In clean wet mounts, the organisms are seen as non-refractile spheres and are acid-fast variable with the modified acid-fast stains; those that are unstained appear as glassy, wrinkled spheres. Modified acid-fast stains show the oocysts as light pink to deep red, and some contain granules or have a bubbly appearance. Oocysts will autofluoresce bright green or intense blue under ultraviolet light. Patients do not respond to antibiotics commonly used for diarrheal treatment such as fluoroquinolones, macrolides, and metronidazole. In otherwise healthy individuals, the disease appears to be self-limiting and may not require treatment other than supportive remedies. In immunocompromised patients or severe disease leading to dehydration, TMP-SMZ, one double strength tablet four times a day is currently the drug of choice. Duration of treatment depends on immune status, and

in severely immunocompromised patients, chronic suppressive therapy may be necessary until immune function recovers.

F. *Sarcocystis* spp.

Sarcocystis spp. include the organism once known as *Isospora hominis* as part of their life cycle. Two well-described species are *Sarcocystis bovi-hominis* (bovine) and *S. suihominis* (porcine). When raw or poorly cooked meat from infected animals is ingested by a human host, gamogony occurs within the intestinal cells and leads to the production of sporocysts in the stool. Humans who ingest meat containing mature sarcocysts serve as definitive hosts. Fever, severe diarrhea, abdominal pain, and weight loss from infection in immunocompromised hosts have been reported, but are relatively uncommon. Eosinophilic enteritis and ulcerative enterocolitis may complicate the course of the disease, especially in severe disease. Humans can also serve as accidental intermediate hosts; however, the sarcocysts that develop in human muscle do not usually cause permanent damage. Some patients occasionally experience fever, myalgia, weakness, and eosinophilia. Symptomatic treatment is usually sufficient and no specific treatment is known to affect the muscle stages of *Sarcocystis* spp. Corticosteroids have been used to treat occasional allergic inflammatory reactions that occur when cysts rupture.

Sporocysts recovered from stool are broadly oval, measuring 9 by 16 μm in size and contain four mature sporozoites and the residual body. Normally, two sporocysts are contained within the oocyst (similar to *C. belli*); however, in *Sarcocystis* infections, the sporocysts are usually already released from the oocyst and normally are seen singly. They are larger than *Cryptosporidium* oocysts, which contain four sporozoites.

For infections in which humans serve as definitive hosts, prevention involves adequate cooking of beef and pork. For infections in which humans are intermediate hosts, careful

disposal of animal feces possibly containing infective sporocysts can minimize risk of infection.

G. Microsporidia

The microsporidia are obligate intracellular parasites that have been recognized in a variety of animals. The organisms found in humans tend to be smaller, ranging from 1.5 to 2 μm long. They are characterized by having spores containing a polar tubule which serves as the extrusion mechanism for injecting the spore content into the host cell. Human microsporidiosis remained rare until the AIDS epidemic. Microsporidiosis is an important emerging opportunistic infection

in HIV-infected patients and appears to have an ever expanding clinicopathologic spectrum among immunocompromised hosts. Severely immunocompromised patients may have concurrent infections causing diarrhea on top of microsporidia, and so response to empiric therapy may be blunted and misleading. To date, nine genera have been recognized in humans (Table 8.4). These are *Enterocytozoon*, *Encephalitozoon*, *Pleistophora*, *Trachipleistophora*, *Brachiola*, *Nosema*, *Vittaforma*, *Microsporidium*, and *Septata*. *Enterocytozoon bieneusi* and the three species of *Encephalitozoon* are the primary microsporidia species associated with human infections. Intestinal microsporidiosis due to *Enterocytozoon bieneusi* causes chronic diarrhea,

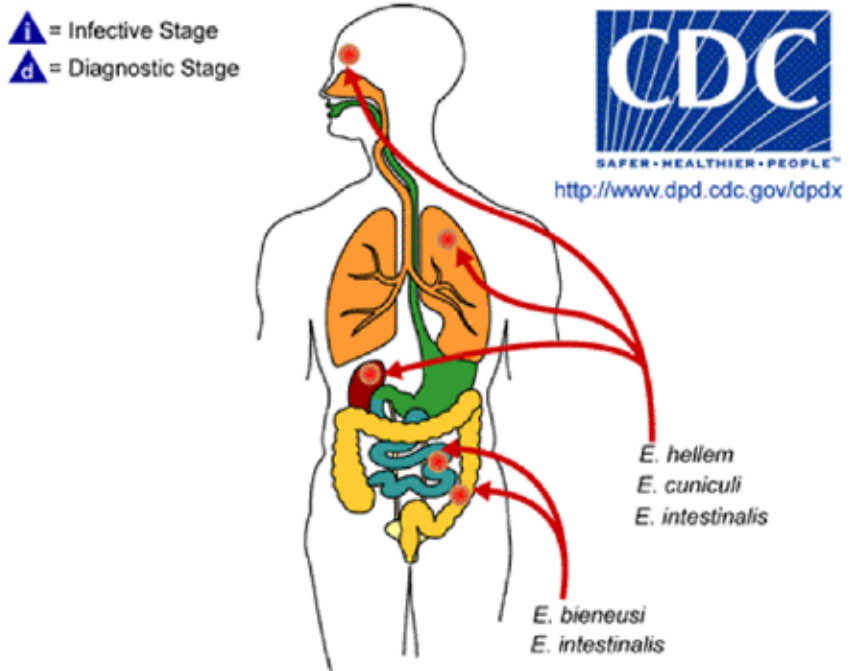
Table 8.4. Microsporidial infections in immunocompromised patients

Microsporidia species	Underlying condition	Clinical syndromes
<i>Enterocytozoon bieneusi</i>	AIDS	Diarrhea, wasting syndrome, bronchitis, pneumonia, cholecystitis, cholangitis
<i>Encephalitozoon cuniculi</i>	AIDS	Keratoconjunctivitis, rhinosinusitis, peritonitis, fulminant hepatitis, seizure
<i>Encephalitozoon hellem</i>	AIDS	Conjunctivitis, keratoconjunctivitis, bronchiolitis, pneumonia, rhinosinusitis, disseminated infection
<i>Encephalitozoon intestinalis</i>	AIDS	Diarrhea, disseminated disease
<i>Pleistophora</i>	AIDS	Myositis
<i>Trachipleistophora hominis</i>	AIDS	Myositis
<i>Brachiola vesicularum</i>	AIDS	Myositis
<i>Nosema ocularum</i>	Non-HIV	Keratitis
<i>Vittaforma cornea</i>	Non-HIV	Keratitis

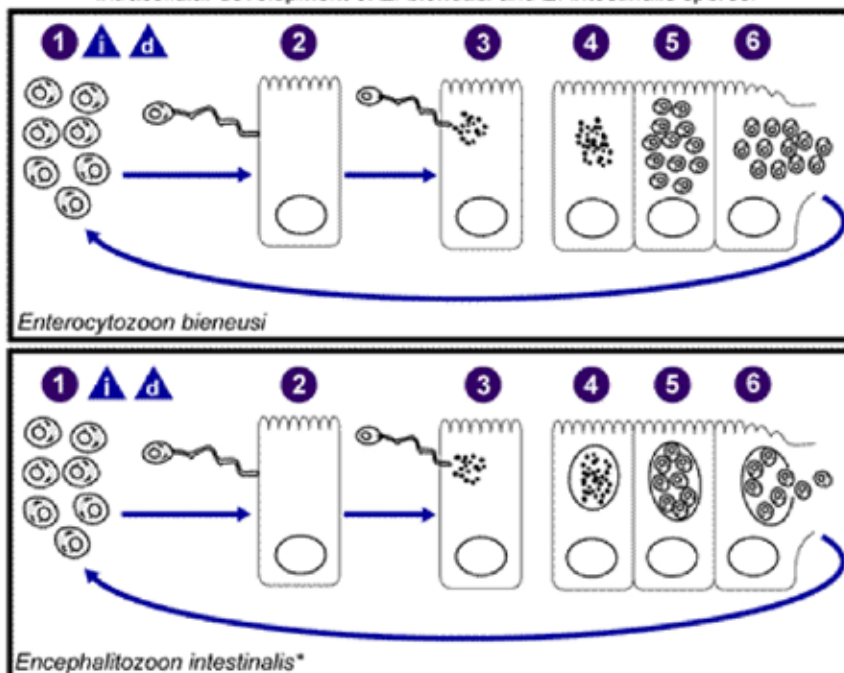
malabsorption, and wasting in AIDS patients. Infections with the other species are rare and sporadic.

The spore is the only life cycle stage able to survive outside the host cell (Figure 8.8). Acquisition of infection is through ingestion of the spores, and once inside the body, single cells are infected by injection of infective sporoplasm through the polar tubule. The microsporidia multiply extensively within a parasitophorous vacuole (genus *Encephalitozoon*) or directly in the host cell cytoplasm (e.g., *E. bieneusi*).

The life cycle includes repeated divisions by binary fission (merogony) or multiple fissions (schizogony) and spore production (sporogony). Both merogony and sporogony can occur in the same cell at the same time. During sporogony, a thick spore wall is formed. The spores are released into the intestinal lumen and are passed out with the stool. Spores are environmentally resistant and can then be ingested by prospective hosts. In the immunocompromised, microsporidial infection can lead to overwhelming disease and death.



Intracellular development of *E. bienersi* and *E. intestinalis* spores.



*Development inside parasitophorous vacuole also occurs in *E. hellem* and *E. cuniculi*.

Figure 8.8. Life cycle of microsporidia
 (Accessed from www.dpd.cdc.gov/dpdx)

Diagnosis of microsporidial infections is by demonstration of spores in feces, urine, and other body fluids or within tissues. This may be challenging due to the small spore size and irregular spore excretion. A number of techniques for increasing yield for recovery and identification of microsporidia in clinical specimens are available. The organisms can be identified in routine histologic preparations. The spores take on a refractile gold appearance in formalin-fixed, paraffin-embedded, routine hematoxylin-and-eosin-stained sections. Spores are occasionally seen very well by using the periodic acid-Schiff (PAS) stain, the methenamine-silver stain, tissue Gram's stain, or acid-fast stains. Spores have a small, PAS-positive posterior body, while spore coat will stain with silver. Spores are acid-fast variable.

Microsporidia spores are difficult to appreciate in stool wet mounts for ova and parasites. Chemofluorescent agents such as Calcofluor White 2MR, Fungi-Fluor, or Uvitex 28 increase sensitivity but may bind non-selectively to debris and cause false positive results. Use of antisera conjugated with fluorescent reporters in detecting spores in clinical specimens increases specificity. PCR methods are available as research tools and may be useful as adjuncts for diagnosis in persistently negative clinical specimens when clinical suspicion remains high. *In vitro* cell culture remains the gold standard but is not practical in routine clinical diagnosis. Serologic tests (carbon immunoassay, indirect IFA, ELISA, counterimmunoelectrophoresis, and Western blotting) have been used to demonstrate IgG and IgM antibodies to microsporidia, but are of uncertain utility in demonstrating active infections.

Treatment for ocular, intestinal, and disseminated disease is with albendazole. Itraconazole can also be used to treat ocular, nasal, and paranasal sinus infection caused by *E. cuniculi* parasites when albendazole fails. Other agents that have been tried are metronidazole,

octreotide, primaquine, lomotil, loperamide, and other anti-diarrheal agents. Fumagillin, an antibiotic derived from *Aspergillus fumigatus*, has activity against microsporidia, and solutions applied topically have been used in corneal infections. In a randomized, double-blind, placebo-controlled trial of fumagillin in patients with chronic *E. bienersi* infection, clearance of microsporidia occurred in all six of the patients in the fumagillin group, as compared with none in the placebo group. HIV-infected patients should be started on highly active anti-retroviral therapy since this facilitates clearance of infection.

H. *Strongyloides stercoralis*

Strongyloides stercoralis is potentially one of the deadliest helminthic parasites due to its ability to complete its life cycle entirely within the human body. Autoinfection can dramatically increase the parasite burden of adult. In normal hosts, the autoinfection is manageable through usual immune mechanisms; but abrogation of cell-mediated immunity unbridles this cycle, allowing for overwhelming and fatal infections.

Filariform larvae are the infective stage; and are acquired by skin contact with fecally-contaminated soil. The larva penetrates intact human skin and sequentially migrates through the heart and lungs, passes up the trachea, is swallowed, and finally grows to maturity in the gastrointestinal tract. Eggs are passed out in the stool, but may hatch before elimination of feces. Non-infective rhabditiform larvae may transform to infective filariform larvae while still in the gastrointestinal tract or on the perianal surface. These can then penetrate the bowel wall or skin and reinitiate the life cycle. This cycle can occur asymptotically at a very low level over many years except for a mild eosinophilia. Immunocompromised patients who have previously been infected with *Strongyloides* or who acquire new infection are at risk for hyperinfection. Development or exacerbation of gastrointestinal and pulmonary symptoms

with detection of increased numbers of larvae in stool and/or sputum is the hallmark of hyperinfection. Among the conditions that may trigger hyperinfection are AIDS, glucocorticoid treatment, and Human T-lymphotropic virus type 1 (HTLV-1) infection.

Glucocorticoids are strongly associated with transforming chronic strongyloidiasis to hyperinfection. Aside from the decrease in cell-mediated immunity, corticosteroids increase the production, mainly in the intestinal wall, of ecdysteroid-like substances which may act as molting signals and increase production of auto-infective larvae.

Patients who have developed severe systemic *S. stercoralis* infections include those with hematologic malignancies, connective tissue disease such as systemic lupus erythematosus, solid organ transplant recipients, and other underlying immunosuppressive conditions. When migrating larvae increase in numbers, abdominal complaints and repeated episodes of unexplained bacteremia or meningitis with enteric bacteria may occur. This is likely due to larval penetration of the bowel leading to translocation of bowel flora into the bloodstream either from the sites of microperforation, attached to the larva, or excreted by the larvae in circulation.

Diagnosis of *Strongyloides* infection is best made by detecting rhabditiform larvae in concentrates of multiple stools. Single stool exam may miss up to 70% of cases; while three stool samples increases diagnostic sensitivity to 50% and seven serial stool samples raises sensitivity to more than 90%.

S. stercoralis resides in the duodenum, making recovery of the larvae in the stool difficult in patients with low worm burden. Ancillary techniques like the Entero-Test string capsule and the duodenojejunal aspiration may increase yield. Other techniques for recovering *Strongyloides* larvae include the Harada-Mori and petridish culture techniques. ELISA to detect *Strongyloides* antibody is 88% sensitive

and 99% specific, however infections with filariae or *Ascaris* can lead to false-positives results and does not distinguish active from past infections.

In disseminated strongyloidiasis, filariform larvae can be found in stool samples as well as sputum, bronchoalveolar lavage fluid, pleural fluid, peritoneal fluid; and surgical drainage fluid. The typical rhabditiform larvae of *S. stercoralis* are characterized by short buccal capsule with an open mouth and the presence of a conspicuous genital primodial packet of cells. Extreme care should be taken when working with materials from a patient suspected of having strongyloidiasis because of possible filariform larvae in the specimen. Gloves should be worn to prevent skin penetration by these larval forms.

Thiabendazole is the drug of choice in both uncomplicated and disseminated infections, but due to potentially severe side effects, alternative chemotherapy with ivermectin and albendazole can be attempted. In a prospective, randomized, open-labelled study comparing a seven-day course of oral albendazole 800 mg day versus a single oral dose of ivermectin 200 µg, cure rates were 38.1% and 76.2%, respectively. In a different randomized trial in rural Zanzibar, a single dose of 200 µg/kg of ivermectin and 400 mg/day for 3 days of albendazole in 301 children with *Strongyloides stercoralis* resulted in cure rates of 83% and 45%, respectively. In another open randomized study of 60 patients with *Strongyloides stercoralis* infection treated with albendazole 400 mg/day for 3 days or ivermectin 150 to 200 µg/kg single dose, parasitological cure with the former was 38% and 83% for the latter.

The efficacy of therapy should be monitored with serial examinations until a negative stool or upper small bowel fluid is obtained. Treatment failure and relapse are not infrequent. In patients with the hyperinfection syndrome, case fatality rates are high (up to 87%) despite appropriate anthelmintic therapy due to the concomitant

immunosuppression and bacteremia. Detection and eradication of *Strongyloides* infection prior to initiation of immunosuppressive therapy is important in preventing the occurrence of disseminated strongyloidiasis.

Other Parasitic Infections

Entamoeba histolytica, the cause of amebic dysentery and amebic liver abscess, infects a large number of people throughout the world. Morbidity and mortality due to *E. histolytica* varies, depending on the geographic area, organism strain, and patient immune status. In patients with intestinal disease, symptoms range from minimal to acute or chronic amebic colitis. Extraintestinal infection occurs when the organisms invade the mucosal lining and are carried via the bloodstream to the liver. *E. histolytica* infection in an immunocompromised host can lead to a higher risk of extraintestinal disease. AIDS patients in endemic areas are at high risk for severe infection.

Blastocystis hominis, a common commensal in the colon, is considered a non-pathogenic intestinal protozoan. However, in the absence of other parasites, bacteria or viruses, it has been known to cause diarrhea, and constitutional symptoms in immunocompromised hosts. *B. hominis* is the most frequently detected parasite among adults; including immunocompromised patients, institutionalized psychiatric or elderly subjects, immigrants from developing countries, and travelers to developing countries. In the same study population, *B. hominis* showed a significant correlation with gastrointestinal symptoms only when detected in subjects with severe immunodepression. Its role as an opportunistic parasite has been described among HIV-infected patients, with a prevalence of up to 52% among in this population.

Free-living ameba can cause severe disease in immunocompromised individuals. Granulomatous amebic encephalitis (GAE) caused by *Acanthamoeba* spp. and *Balamuthia mandrillaris* occurs as a subacute or chronic

disease with focal granulomatous lesions in the brain. Conditions associated with GAE include amebic keratitis, skin ulcers, liver disease, pneumonitis, diabetes mellitus, renal failure, rhinitis, pharyngitis, and tuberculosis. Predisposing factors include alcoholism, pregnancy, SLE, hematologic disorders, AIDS, chemotherapy, radiation therapy, and steroid treatment. *Acanthamoeba* spp. are now well-described as opportunistic pathogens in AIDS patients, particularly those with a low CD4+ cell count. Unfortunately, the diagnosis of this rare infection requires a high index of suspicion, since both clinical and histological findings may mimic those of disseminated fungal or algal disease. Clinical manifestations of AIDS patients infected with *Acanthamoeba* include non-specific systemic complaints such as fever and chills, nasal congestion, neurologic symptoms, and musculoskeletal and cutaneous lesions. Some patients may develop erythematous nodules, chronic ulcerative skin lesions, or abscesses. Over 100 cases of GAE caused by *Acanthamoeba* spp. have been recorded worldwide and 53 of these occurred in AIDS patients in the United States. Although *Acanthamoeba* infection typically stimulates granuloma formation, the response in AIDS patients is minimal or absent due to severe immunosuppression.

The leptomyxid ameba *Balamuthia mandrillaris* is uncommon and was previously thought to have no pathogenic potential. *B. mandrillaris* is very similar to GAE and has an unknown incubation period. Its clinical course tends to be subacute to chronic. There have been over 74 cases of *B. mandrillaris* GAE reported worldwide, 11 of whom were AIDS patients in the USA. *Giardia duodenalis* is a parasitic flagellate commonly found in many parts of the world. *Giardia* infection generally manifests as intestinal diarrhea. Infection in healthy hosts is usually self-limited, but may contribute to morbidity from diarrhea especially in malnourished children and the

elderly. Inadequate sanitation is a major risk factor for acquisition of giardiasis, and drinking of contaminated water is the usual mode of infection to travelers in developing countries. AIDS patients presenting with diarrhea should be screened for giardiasis. Trophozoites can be seen on wet mounts and are better seen with Giemsa staining. Lateral flow assays that detect antigen in stool are commercially available and are usually combined with *Cryptosporidium*. While treatment of giardiasis in healthy hosts is straightforward with metronidazole or tinidazole, those who are severely immunocompromised may require longer duration of treatment and may have more frequent relapse.

Epidemiological studies also suggest that malaria is a deadly co-factor for AIDS. The results of Ugandan study by Whitworth, et al. involving 484 participants making 7,220 clinic visits between 1990 and 1998 did show an increased frequency of clinical malaria (2.0%) and parasitemia (11.8%) associated with HIV-1 infection as opposed to their HIV-negative counterparts, 0.7% and 6.3%, respectively. Lower CD4 cell counts were associated with increased parasite densities and increased risk of clinical malaria. In addition, infants born to mothers co-infected with HIV and malaria had a four-fold higher mortality rate than infants born to mothers infected with either HIV or malaria alone.

There is considerable geographical overlap between malaria and HIV and increasing evidence on a direct link with one disease making the other worse and more difficult to treat.

Malaria and HIV/AIDS are two of the most important infectious diseases worldwide, accounting for almost 9% of the total burden of disease in sub-Saharan Africa (Figure 8.9). Malaria and HIV cause more than four million deaths a year combined, and are both concentrated primarily in sub-Saharan Africa, Asia, and South America. More than 500

million cases of malaria occur every year, at least a million of which cause deaths. An estimated 30 to 36 million people are living with HIV in Africa, resulting in more than 3 million deaths every year. Malaria is more common and severe in adults with HIV, pregnant women, and children.

Guidelines for treatments of the two infections are often conflicting. There are also issues around drug resistance and cross-reactions between drugs, as well as concerns that some medications used to treat HIV-positive persons could be harmful for malaria treatment in certain settings.

HIV not only increases the incidence and severity of malaria, it also compromises malaria treatment. HIV infection can decrease the response to standard antimalarial treatment. For HIV-positive adults with a weakened immune system (a low CD4 count), antimalarial drugs are less likely to be effective. Malaria contributes to an increase in viral load among HIV-positive people which can potentially accelerate the progression from HIV to AIDS.

In a prospective, cross-sectional study, in the Central Hospital of Maputo, Mozambique last October 2006, risk factors for fatal outcome were determined and impact of HIV on the accuracy of malaria diagnosis was assessed. Among 333 included patients, 15% (51/333) had "presumptive malaria," 10% (28 of 285 tested persons) had positive malaria blood slides, while 69.1% (188/272) were HIV positive. Seven percent (n=23) had confirmed malaria, after the diagnosis was rejected in patients with neck stiffness or symptom duration longer than two weeks (n=5) and persons with negative (n=19) or unknown malaria blood slide (n=4). Clinical stage of HIV infection, hypotension, and hypoglycemia were associated with fatal outcome. The study suggests that the fraction of febrile illness attributable to malaria is lower in HIV positive adults. HIV testing should be considered early in evaluation of patients with suspected malaria.

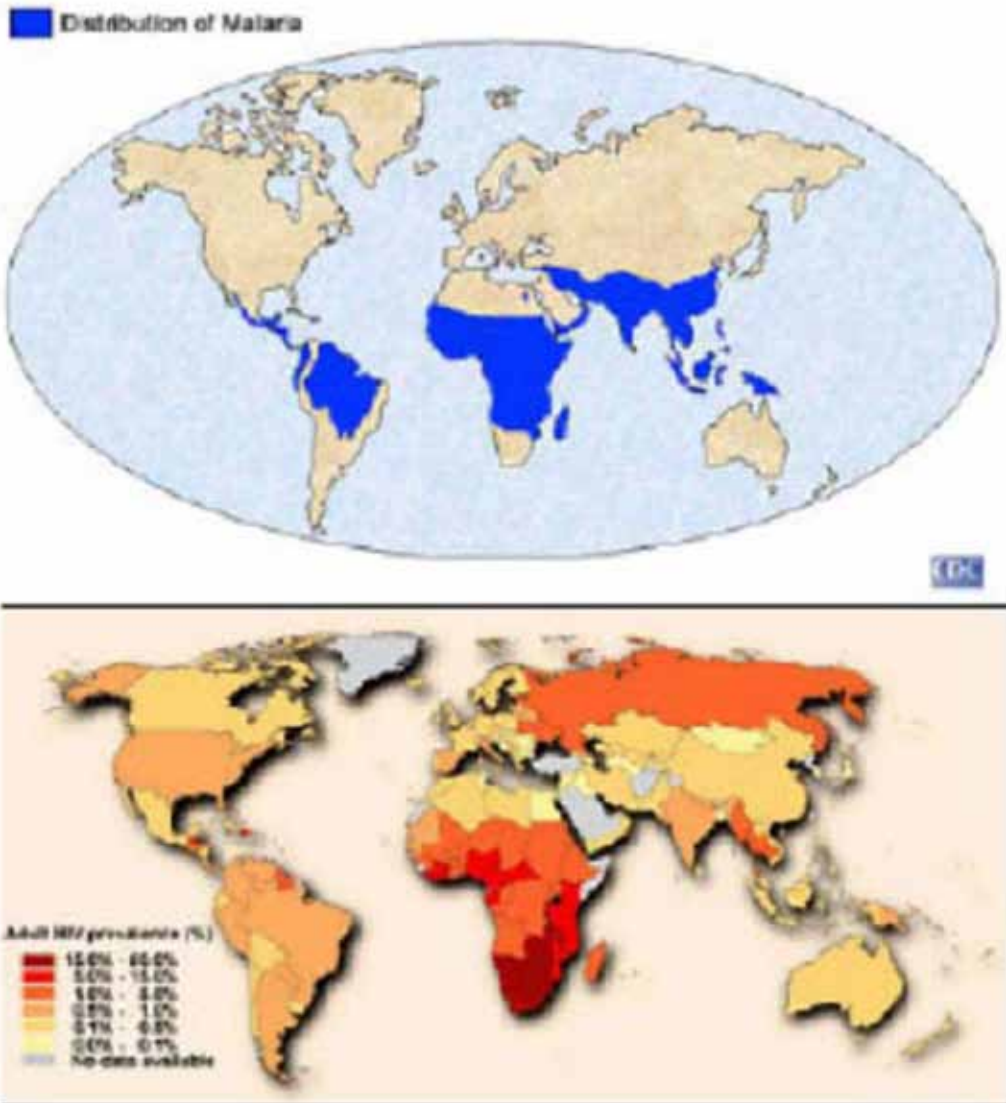


Figure 8.9. Distribution of malaria
(Accessed from www.cdc.gov)

Superimposed endemic parasitic infections in tropical countries present a major health problem among HIV-infected individuals and malnourished hosts. Non-opportunistic intestinal parasites such as hookworms, *Opisthorchis viverrini*, and *A. lumbricoides* are common regardless of HIV status. In a prospective observational study on intestinal

parasitic infections among HIV infected and uninfected children with diarrhea in Thailand, intestinal parasites were identified in the stool specimens of 27 out of 82 (33%) HIV infected children and were significantly higher than the uninfected children [12 out of 80 (15%)]. In Africa faster progression to AIDS and increased HIV viral load occurred in areas

highly endemic for helminths. These long-lasting parasitic infections cause widespread activation and dysregulation, inducing a dominant Th2 cytokine immune profile and an immune hyporesponsive state. Helminths induce a predominantly Th2 response, and this has been associated with progression of HIV. Endemic tropical non-opportunistic parasitic infections present a special and significant risk in immunocompromised individuals. It is important for the clinicians and laboratory personnel to be aware of the problems these organisms can cause and recognize their clinical relevance.

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Neglected Tropical Diseases

Vicente Y. Belizario, Jr., Amelia M. Breyre, Francis Isidore G. Totañes

Neglected Tropical Diseases (NTDs) are a biologically diverse group of chronic diseases unified by their strong association with poverty. NTDs are caused by a disparate group of pathogens, including viruses, bacteria, protozoa, and helminths. Their modes of transmission also vary tremendously; some are parasitic diseases that spread through hosts and vectors (e.g., fish, snails, mosquitoes, etc.), while others are transmitted through water. All NTDs, however, share several of the following features: (a) they affect populations with low visibility and little political voice; they are

relatively neglected by research; (b) they have an important impact on morbidity and mortality; and (c) they can be prevented or possibly eliminated using effective and feasible strategies.

NTD Disease Distribution

Internationally, more than a sixth of the world's population suffer from one or more NTDs. The distribution of these diseases varies tremendously regionally. Figure 8.10 is a map that shows the global distribution and overlap of the most common NTDs. One-third of the global prevalence of intestinal helminthiasis, and

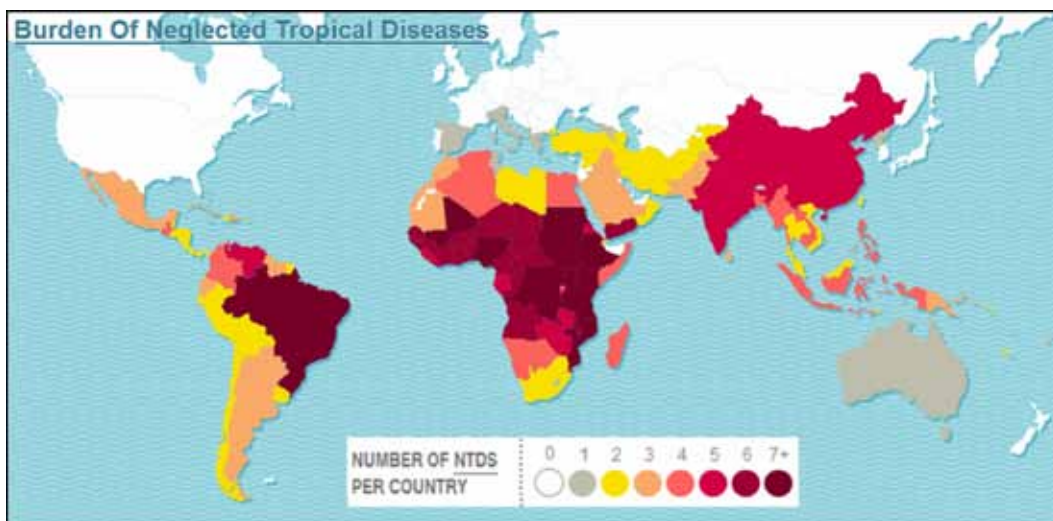


Figure 8.10. Global distribution of neglected tropical diseases (NTDs) by number of NTDs per country
(Accessed from <http://ffctn.com/a/ghs-ntd>)

a majority of foodborne trematode infections can be found in Southeast Asia and China. Approximately one-half of the active trachoma infections, and a significant proportion of the number of cases of lymphatic filariasis (LF) and

schistosomiasis are also in this region. Table 8.5 enumerates the NTDs targeted by the World Health Organization (WHO) for control, with a focus on those endemic to the Philippines.

Table 8.5. Neglected tropical diseases targeted by the WHO

Disease	Endemic in the Philippines
Buruli ulcer	
Chagas disease*	
Cysticercosis*	✓
Dengue	✓
Dracunculiasis (guinea-worm disease)*	
Echinococcosis*	
Fascioliasis*	
Human African trypanosomiasis	
Leishmaniasis*	
Leprosy	✓
Lymphatic filariasis*	✓
Onchocerciasis*	
Rabies	✓
Schistosomiasis*	✓
Soil-transmitted helminthiasis*	✓
Trachoma	
Yaws	
*Diseases caused by parasites	

Burden of Neglect

NTDs disproportionately affect the poorest and most marginalized, including the rural poor, residents of urban slums, out of school youth, women, and indigenous people whose access to formal health services are limited for cultural, social, or geographic reasons. It is difficult to quantify the social burden associated with crippling disabilities and reductions in productivity of individuals and communities caused by NTDs. Nevertheless, efforts to measure the social and economic impact of NTDs can provide an understanding of the extent of disease burden, and are important in order to guide policies and prioritize disease control programs. Calculations of disability rate, for example, estimate that a total of 45.4 days off-work lost per infected person/

year is attributable to schistosomiasis in the Philippines.

The concept of disability-adjusted life years (DALYs) was developed to quantitatively assess the burden of individual diseases. DALYs take into account both premature mortality (years of life lost) and disability (years of life lived with a disability weighted by the severity of the disability). DALYs assigned to a specific disease at a particular time gives the estimated sum of years of potential life lost due to premature mortality and years of productive life lost. For example, it is estimated that 5,941,000 years of potential life are lost globally due to lymphatic filariasis. The use of DALYs, however, is somewhat controversial since its design contains inherent systematic flaws that result in under evaluation of the importance of chronic diseases such as NTDs. Because DALYs focus more on individual risk rather than the ecology of the disease, the weight of disability for chronic diseases in the context of poverty tend to be underestimated.

The concept of a quality-adjusted life years (QALYs) is an alternative means to quantify losses attributable to disease. The QALYs system uses estimates from preference-based health related quality-of-life interviews administered to groups of patients or to members of the general population in an endemic community. QALYs are better able to assess the societal context of disease impact that may not be accurately captured by DALYs. Improvement of DALYs calculations and development of new metrics such as QALYs are ongoing. Such efforts are an important aspect of assessment of the burden of NTDs because they provide a mechanism for determining health priorities.

Polyparasitism

The burden of NTDs is further compounded by the fact that infection with multiple parasite species, known as polyparasitism, is more often the norm rather than the exception. Community

surveys in Cote d'Ivoire demonstrated infection with at least two intestinal parasites (*Schistosoma mansoni*, soil-transmitted helminths and/or intestinal protozoans) in 90.2% of the sampled population. In Brazil, co-infection with *Necator americanus* and *S. mansoni* was observed in 41.0% of the community participants examined. In a community survey in China, 27.8% of those surveyed were infected with at least two parasite species (*Ascaris*, *Trichuris*, and/or *S. japonicum*).

Local sentinel parasitologic surveys on school-age children revealed multiple infections with at least two helminths (soil-transmitted helminths, *Schistosoma japonicum* and/or heterophyids) in 20.4% of those examined. Similarly, co-infections between different STH species and *S. japonicum* were observed in 13.1% of school-age children in indigenous peoples in Davao del Norte.

Although there are existing data on the global prevalence and burden of individual parasitic diseases, there are still no accurate estimations on the global burden of polyparasitism. Estimates of populations at risk of multiple parasitic infections have been described by looking into co-distribution rather than co-infection. Currently, there are limited studies on the epidemiology and impact of polyparasitism. Research looking into the use of polyparasitism as a parameter for effective disease control needs to be explored.

Risk Factors for Polyparasitism

The risk for polyparasitism, as with individual infections, is influenced by the combined effects of several factors. Intrinsic factors are attributed to host resistance that is influenced by age and sex; and linked to frequency of exposure to infection, as well as development of immunity, or a combination of both. *Ascaris* and *Trichuris* infections, for example, are most prevalent among the 5 to 15 years old age group. Hookworm infections are most prevalent among middle-aged individuals

(15-24 years old), and are more common in males. A community parasitologic survey in Cote d'Ivoire observed the highest frequency of polyparasitism among adolescents and young adults (15-24 years old).

Geographic distribution, in relation to the overlapping of areas of endemicity, also contributes to the occurrence of polyparasitism. In addition, behavioral factors may also be attributed to polyparasitism. Behavior related to personal hygiene can greatly contribute to infection of parasites with similar modes of transmission. Socioeconomic status, living conditions and access to health and sanitary facilities also influence the distribution of polyparasitism and parasitic infections in general. Individuals of lower socioeconomic status are less likely to have adequate water and sanitation, and are less likely to invest in bed nets for protection against mosquito-borne diseases. Similarly, low education levels have been associated with limited access to effective treatment, and less compliance with preventive measures.

A study by Ellis et al. that looked into environmental and genetic predispositions to polyparasitism revealed that the risk of *Ascaris* and *Trichuris* co-infection, and *S. japonicum* and *Trichuris* co-infection were significantly influenced by environmental or household conditions. Data from this study also revealed that there is a significant genetic component attributed to the risk of multiple parasitic infections. This suggests that polyparasitism may aggregate in a familial pattern.

Combined Impact of Polyparasitism

Infection with multiple parasites intuitively results in higher morbidity than the impact of a single infection. Malnutrition, as exemplified by wasting and stunting, arises as a result of co-infections with malaria, STH, and/or *Schistosoma*. Intestinal helminth infections cause intestinal inflammation and reduced food intake, while malaria and schistosomiasis

may trigger inflammatory cytokines that cause anorexia and induce a catabolic response.

Anemia in malaria infection is from hemolysis and phagocytosis, while anemia from STH infections arises from chronic intestinal blood loss. A local study has demonstrated a significant association between anemia and *S. japonicum* infection. Given the different mechanisms by which these infections bring about malnutrition and anemia, it is possible that the effects of co-infection on malnutrition and anemia are additive. Studies in Kenya revealed significantly lower hemoglobin among preschool and school age children with malaria-hookworm co-infections, compared to those with single infection. Another study done in Nigeria has shown lower mean hemoglobin among pregnant women with co-infections with malaria and STH, although the difference was not statistically significant.

An increasing number of studies have demonstrated significant associations between co-infections with different helminth species. Helminth infection has been shown to elicit an immune response that either results in the production of non-cytophilic antibodies allowing increased susceptibility to further infection, or results in effective inflammatory factors that offers protection against other parasitic infections.

A notable increase in hookworm intensity has been described with an increasing number of co-infecting helminths (*Ascaris* and *S. mansoni*). With regard to *Ascaris* infection, there was a significant increase in intensity of infection in the presence of hookworm co-infection, and a significant decrease in the presence of *S. mansoni* co-infection. The synergistic effect of hookworm infection with other helminth infections may be attributed to immunomodulation resulting in reduced cellular reactivity. T-regulatory cells (Tr1) that secrete cytokines may play a role in the down-regulation of the host's immune response to subsequent helminth infections, thus resulting in greater intensities of infection.

A synergistic effect has also been demonstrated between *Ascaris* and *Trichuris* infections, while protective effects against malaria have been reported as a result of *Ascaris* or *S. haematobium* infections.

Strategic Approaches

A. Disease Surveillance

Successful control of NTDs requires active surveillance programs at the local level in order to understand prevalence and disease distribution. Information on the burden of NTDs is important to determine specific disease control and prevention strategies. On the other hand, data on the geographical distribution of NTDs can help direct resources to priority areas, especially in low-income countries where NTDs are prevalent and resources are limited.

Strengthening the capacity of health professionals is important for early diagnosis and treatment of cases. The local medical technologist plays a major role in the performance of appropriate and accurate laboratory examinations. Accurate and timely diagnosis will not only contribute to the proper treatment and early prevention of morbidity, but also limit under- or over-reporting of cases. This will also result in reporting of reliable data for proper disease monitoring and surveillance.

B. Preventive Chemotherapy

The WHO defines preventive chemotherapy as a major strategy for the control of a number of parasitic diseases through morbidity and transmission control. Preventive chemotherapy through mass drug administration is recommended for the control of lymphatic filariasis, onchocerciasis, schistosomiasis, and soil-transmitted helminthiasis. Given the overlapping distribution of many NTDs, the WHO recommends combined control strategies in a drug-based rather than a disease-based approach. The drug-based approach looks into combined control of diseases that are targeted by the same drugs.

Co-administration of praziquantel and albendazole, as well as co-administration of ivermectin and albendazole are recommended for use in mass treatment strategies in co-endemic areas. Initial studies on the co-administration of albendazole, praziquantel, and ivermectin have shown no clinically significant pharmacokinetic interactions when given together to healthy volunteers. These suggest that co-administration of the three drugs is not expected to yield additional adverse reactions; however, it is important to consider precautionary measures when administering drug combinations to infected individuals.

Infrastructural interventions: Since NTDs are strongly associated with poverty, many of those afflicted tend to have limited access to clean water and proper sanitation facilities. Access to clean water is necessary in order for any control program to have a lasting impact on reducing transmission. Improvements in infrastructure need to be supplemented with educational programs to increase awareness and to promote habits that reduce transmission, such as proper hygiene.

C. Integrated Control

Because many of the NTDs are parasitic, control of these diseases share similar intervention strategies. They can be integrated into a streamlined control program in order to increase efficiency and reduce costs. Integration of health programs should consider capitalizing on existing infrastructure and programs. Targeting groups that are at high-risk for multiple infections, such as pre-school children, school-age children, and farmers; as well as groups at high risk for morbidity, such as pregnant and adolescent females, should be considered in integrating NTD control programs. These groups can be reached through the same channels, such as existing health and education systems with extended community-based coverage where populations

are severely affected and underserved. For example, combined control of schistosomiasis and STH infections may be conducted through deworming of school children as part of the school health and nutrition program.

Recognizing the importance of local health systems, collaboration between the health and education sectors, as well as the local government units are important for a more unified and concerted approach to the control of parasitic infections in the community. Future operational researches on combined control strategies and their impact on the prevalence of NTDs and associated morbidities, as well as cost-benefit studies will be important in establishing evidence-based guidelines for effective disease control.

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Preventive Chemotherapy

Vicente Y. Belizario, Jr., Francis Isidore G. Totañes, Paul Lester C. Chua

Neglected tropical diseases (NTDs) occur most commonly in the setting of extreme poverty, especially among the rural poor and disadvantaged urban populations. Four of the most prevalent NTDs are due to helminths which are lymphatic filariasis (LF), onchocerciasis, schistosomiasis, and soil-transmitted helminthiasis (STH). Epidemiological studies reveal a broad geographic overlap among these diseases, especially among impoverished populations with limited access to health services and sanitation. The focus of public health interventions against these helminth infections has transformed over the years; from measures targeting extra-human stages of the life cycle of the worms, such as vector control or environmental sanitation; to measures targeting the human host, specifically through treatment with the use of anthelmintics at regular intervals. The control of these neglected diseases is considered a vital step towards achieving the majority of the eight Millennium Development Goals, but despite the availability of low-cost and effective public health interventions, a large number of the world's poorest individuals remain affected with these diseases.

The main strategy, recommended by the World Health Organization (WHO) for controlling these infections, is the implementation of large-scale preventive chemotherapy (PCT) among population groups at risk. PCT is the regular, systematic, large-scale intervention involving the administration of one or more anthelmintics to selected population groups, with the aim of reducing morbidity and transmission of selected helminth infections.

Characteristics of PCT

Three key characteristics determine PCT as a public health intervention.

First, PCT utilizes population-based diagnosis in assessing the burden of helminth infections in a population through rapid community assessments and/or sentinel surveys applied to a sample of its individuals. Surveys can utilize appropriate diagnostic tests or standard questionnaires screening for symptoms or signs, or for behaviors associated with risk of infection. Population-based diagnosis can also be carried out retrospectively by analyzing existing epidemiological data. Based on results of parasitologic assessment, an appropriate intervention is selected. Population-based diagnosis distinguishes PCT from the clinical approach in which diagnosis is performed at the individual level prior to treatment.

Second, PCT implements population-based treatment through large-scale delivery of single-administration drugs by non-medical personnel (e.g., teachers, volunteers, or community drug distributors), and the use of non-medical settings (e.g., schools, *barangay* halls, churches) as fixed points for drug distribution (Figure 8.11). On the other hand, personalized case management treatment is performed by



Figure 8.11. School teachers administering deworming tablets to students in a public elementary school in Biñan, Laguna (Courtesy of Dr. Vicente Belizario, Jr.)

specialized personnel on individuals reporting to health facilities.

Lastly, PCT is implemented at regular intervals based on the parasitologic status as determined by the population-based surveillance. The intervention is repeated without the need for further diagnostic interventions, although implementation of a monitoring system is important.

Modalities of Implementation

There are three modalities by which PCT interventions can be implemented.

- Universal treatment is the administration of anthelmintics to the entire population of an area (e.g., state, region, province, district, sub-district, village) at regular intervals, irrespective of the individual infection status.
- Targeted treatment is the administration of anthelmintics at regular intervals to specific high-risk groups in the population, defined by age, sex, or other social characteristics (e.g., school-age children, farmers), irrespective of the individual infection status.
- Selective treatment is the administration of anthelmintics to all infected individuals (confirmed or suspected) who are identified after a regular parasitologic screening of a population group living in an endemic area.

Currently Targeted Diseases

PCT targets four NTDs (LF, onchocerciasis, schistosomiasis, and STH) because of a number of reasons. First, helminths responsible for the four diseases are unable to replicate in humans and require one or more obligate passages outside the host (e.g., in an intermediate host, in a vector, or in the environment) in order to

complete their life cycle. Consequently, direct human-to-human spread is unfeasible and disease transmission becomes a slow process. These facts suggest that the rate of increase in number of worms within a human host that contributes to the intensity of infection is slow depending on subsequent re-infection episodes. The risk of developing morbidity and the likelihood of disease transmission are dependent on the individual's intensity of infection. As the intensity of infection increases slowly, the individual's risk of developing morbidity also increases slowly, explaining why early-stage manifestations associated with the targeted helminth infections are frequently overlooked. Second, community diagnostic procedures are available for each of the four diseases. Third, drug delivery strategies relying on resource persons based in schools or within communities have been developed; and lastly, recommended anthelmintics are low cost or given by pharmaceutical companies as donations. All these factors contribute to contain costs and make the PCT interventions feasible for implementation against the four target diseases.

In addition, all anthelmintics currently used in PCT interventions [albendazole (ALB), diethylcarbamazine (DEC), ivermectin (IVM), mebendazole (MBD), and praziquantel (PZQ)] are safe (i.e., adverse events are rare, mild, and transient), and therefore appropriate for use in interventions targeting infected, as well as non-infected individuals. Temporary minor reactions following treatment occur mainly as a result of the body's response to the dying worms. Thus, heavily infected individuals are expected to experience the most reactions. In general, the number of individuals reporting for adverse reactions is highest during the first round of treatment and tends to decrease during succeeding rounds.

Such effective anthelmintics are also simple to administer allowing the drug distribution by non-medical personnel possible. In the War on Worms—Western Visayas approach, a local

government unit (LGU) led, school-based, school teacher-assisted mass drug administration (MDA) has resulted in significant reductions

in the prevalence and intensities of infection among the school children after two years of implementation (Figure 8.12).

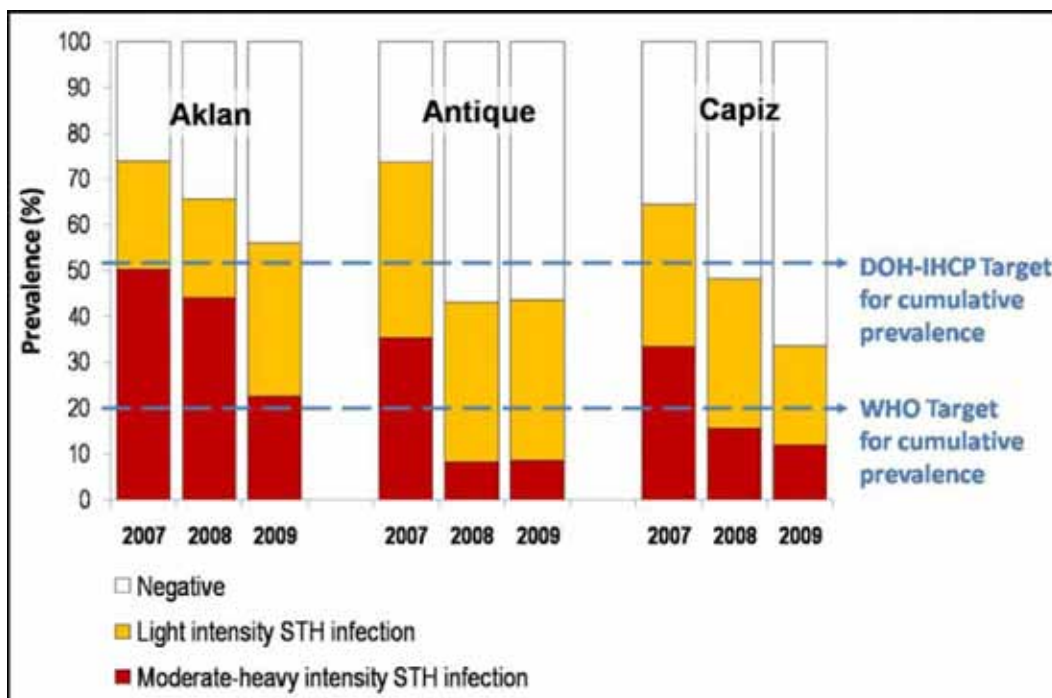


Figure 8.12. Cumulative STH prevalence and heavy intensity infections in school-age children in Aklan, Antique, and Capiz, 2007-2009
(Courtesy of Dr. Vicente Belizario, Jr.)

The following general precautionary measures are recommended to ensure the safe implementation of large-scale drug delivery:

- Seriously ill individuals, who are unable to engage in normal activities of daily living without assistance, should be excluded from large-scale treatment;
- Program managers must ensure that targeted individuals for drug administration are adequately informed about the possible adverse reactions and necessary interventions in the event of such reactions;
- Program managers must ensure the availability of medical care and support for individuals who may experience adverse reactions during rounds of treatment;
- Any serious adverse reactions should be carefully recorded and relayed to the appropriate authorities;
- Individuals who have previously suffered rare serious adverse reactions caused by the drugs should be excluded from treatment;
- Scored tablets should be broken into smaller pieces or crushed before administration to young children to prevent choking or asphyxiation; and
- Program managers should be aware of other MDA for other diseases in the

same area. This is to minimize the risk of targeted individuals suffering from adverse reactions due to interactions between drugs distributed by different programs.

In the Philippines, three helminth infections are targeted for control or elimination by the Department of Health through national programs that utilize MDA as a major strategy (Table 8.6).

Table 8.6. Target population, drug recommended, and mass drug administration frequency of health programs in the Philippines

Health Program	Target population	Drug/s recommended	Frequency
Expanded <i>Garantisadong Pambata</i>	Preschool-age and school-age children 0-14 years old	MBD/ALB	Twice a year
Integrated Helminth Control Program	School-age children 6-12 years old	MBD/ALB	Twice a year (January and July)
Schistosomiasis Control Program	5-65 years old (in endemic areas)	PZQ	Once a year (July)
Filariasis Elimination Program	2-65 years old (in endemic areas)	DEC plus ALB	Once a year (November)
Source: Department of Health. Integrated helminth control program: mass treatment guide. Manila (Philippines); Department of Health; 2006. p. 6-21.			

Drug Combinations

A number of studies have investigated the safety of drug combinations in the treatment of helminth infections.

- ALB and PZQ can be safely co-administered for STH and schistosomiasis.
- MBD and PZQ have been widely co-administered in many countries and reported to be safe for STH and schistosomiasis.
- ALB plus DEC is also a safe combination in the treatment of lymphatic filariasis and STH.

The WHO has endorsed the co-implementation of MDA, also referred to as the integrated approach to PCT.

Promise of Integrated PCT

Reports have revealed that many Ministries/ Departments of Health in disease-endemic countries have supported the control of NTDs through independent and parallel programs, with

each maintaining its own planning, funding, drug distribution system, MDA campaign, monitoring, and evaluation. Because of the similarities of program strategies, epidemiologic overlap of targeted diseases among affected populations, and the availability of drugs, these NTD control/elimination programs are suited for an integrated implementation in a way where coordinated MDA interventions for multiple diseases are implemented to reduce the duplication of efforts in treating the diseases separately. Such integration and coordination of program activities among different disease-specific programs should lead to better drug delivery, increased health benefits, and better use of limited resources reaching more affected and at-risk individuals.

Ancillary Benefits and Advantages

Sustained, large-scale PCT against helminth infections results in a number of benefits and advantages:

- Relief from other NTDs (e.g., foodborne trematode infections) and

from ectoparasitic infections (e.g., scabies and lice) with commensurate health benefits;

- Significant increase in weight and height among children leading to improvement of nutritional status and general health;
- Increase in school participation and improvement of school performance in children; and
- Reduced maternal anemia in pregnant women and improved infant birth weight and survival.

Monitoring and Evaluation

Monitoring and evaluation are integral components of any control program and are vital to ensure both effective implementation and maximum benefit for infected individuals, their families, and communities. An appropriate evaluation system allows proper documentation of the program's impact, updates current practices, and guides future program direction. It is important that the outcome of monitoring and evaluation activities (i.e., good practices and challenges) be shared with communities,

relevant government units, and donors to maintain their interest in and support for the program.

Monitoring and evaluation should be carried out with as little expense as possible so as not divert resources away from implementation activities. The WHO recommends that approximately 5 to 10% of the program budget be allocated for monitoring activities.

Monitoring and evaluation are based on the periodic collection and analysis of variables or indicators with the aim of measuring changes occurring during program implementation. The suggested indicators for schistosomiasis and STH control programs in school-age children can be grouped into three categories (Figure 8.13). Process and performance indicators are used for monitoring, and performance and impact indicators for evaluation (Table 8.7).

Drug coverage is the minimum indicator for assessing the performance of large-scale PCT interventions. Coverage refers to the proportion of individuals in the target population or group who have actually swallowed the recommended drug/s.

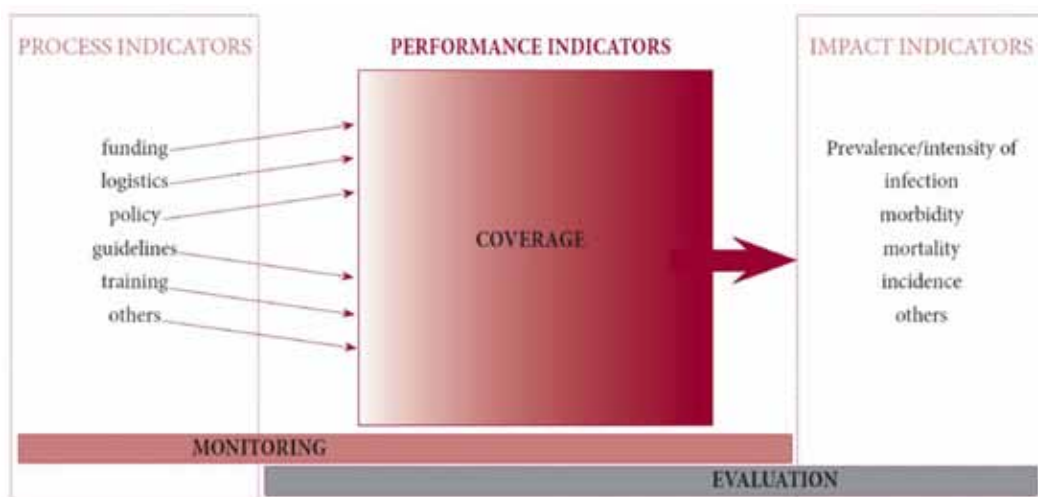


Figure 8.13. Process, performance, and impact indicators for helminth control
(From World Health Organization. Helminth control in school-age children: a guide for managers of control programmes. 2nd ed. Geneva: World Health Organization; 2011.)

Table 8.7. Categories, usage, and frequency of collection of indicators

Category	Use	Frequency of collection
Process	Determine whether organizational elements of the control program are in place and are functioning properly	At every drug administration round
Performance	Assess whether coverage of the control program has reached its objective	At every drug administration round
Impact	Assess whether the health impact of the program has been achieved	At baseline and every 2-3 years thereafter
Source: World Health Organization. Helminth control in school-age children: a guide for managers of control programmes. 2nd ed. Geneva: World Health Organization; 2011.		

Every effort should be made to ensure direct observation of MDA (i.e., administration of the appropriate dose in the presence of the drug provider) (Figure 8.14). If actual swallowing of tablets by targeted individuals cannot be observed directly, random cluster surveys can be undertaken to estimate the actual coverage.

Monitoring drug coverage has several important outcomes.

- Reliable drug coverage rates contribute to accurate information necessary for



Figure 8.14. Checking for tongue discoloration after administration of deworming tablets to school children to ensure compliance (Courtesy of Dr. Vicente Belizario, Jr.)

policy formulation for NTD control and elimination.

- Difficulties encountered during rounds of MDA can be revealed such as the identification of areas where fewer individuals receive drugs than intended.
- Providers of drugs and funds to support drug delivery, including the governments of disease-endemic countries, can be assured that the provided support is cost-effective.
- Workers and volunteers involved in drug delivery can be informed about their efforts, which can contribute to maintaining staff morale.
- Advocacy for more support for NTD control is strengthened by knowledge that many people in need are getting treatment.
- Forecasting for drug supplies for future treatment rounds is supported.

Role of Social Mobilization

These neglected diseases, especially helminth diseases, do not rapidly cause death and are more insidious in nature than many diseases of acute onset. Health care providers therefore consider NTDs a low priority.

The objective of PCT interventions is to ensure that all eligible individuals in affected communities swallow the recommended drugs. This behavioral change is dependent on the acceptance of targeted individuals for treatment, as well as on the health care providers' capacity to adequately inform and motivate the community. Social mobilization is a complex process that involves program implementation, health care delivery services, health care providers, and strategies for mobilization and communication interacting to influence behavioral change in people. Experiences with existing health care programs have shown that this aspect of social mobilization is not given adequate priority during the planning of PCT

interventions. As characteristics of communities and responses to various communications from the health care providers differ, proper understanding is essential in planning effective social mobilization campaigns. Investment in social mobilization strategies is critical in sustaining high drug coverage throughout the duration of health programs (Figures 8.15–8.16).



Figure 8.15. Former DOH Secretary Francisco Duque III and former Antique Governor Salvacion Perez administering anthelmintics to school children in Pandan Central Elementary School, Antique during the launch of the War on Worms—Western Visayas (From War on worms goes to Western Visayas. *Philippine Star*. 2007 Nov 27;Health & Family:E-2.)



Figure 8.16. Parade of school children and teachers during the launch of War on Worms—Biñan, Laguna (Courtesy of Dr. Vicente Belizario, Jr.)

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Emporiatics for the Filipino Traveler

Edsel Maurice T. Salvaña, Arthur Dessi E. Roman

The past years have shown an exponential increase in the amount of local and international travel. The United Nations' World Tourism Organization reported that international tourists numbered around 922 million in 2008. This number is expected to exceed one billion in 2010 and 1.6 billion by 2020. Not only are more people traveling, previously inaccessible areas are now being explored due to increased technology and urban sprawl. Locally, the Department of Tourism reported that tourist volume grew by 6.64% in the Philippines' top destinations during the first quarter of 2010, with foreign tourist arrivals increasing by 7.89%, while domestic tourism increased by 6.09%. Metro Manila remains the leading destination, while Cebu and Camarines Sur follow.

Several factors have contributed to the rise in domestic and foreign travel. Among these are socio-cultural factors such as the recognition that travel is a highly desirable activity which expands one's knowledge and outlook in life, more favorable economics of travel including lower transport and travel costs, higher disposable incomes, built-in leave as part of employment benefits and perks, simplification of logistics in terms of availability of online facilities for arranging travel, ecotourism, medical tourism, and all-inclusive packages.

In 2008, more than half of all international arrivals were motivated by leisure, recreation, and holiday travel, while business accounted for another 15%, and 27% was due to other purposes such as visiting friends and family, religious travel, and medical tourism among others. More than half (52%) arrived via air transport, while the rest used surface transport

(48%) by road (39%), rail (3%), or over water (6%).

This large increase in travel activity, coupled with the threat of various geographically-associated emerging diseases including influenza A (H1N1), avian flu (H5N1), and the severe acute respiratory syndrome (SARS), makes emporiatrics or travel medicine increasingly relevant. The various risks that travel poses to health can be challenging to prevent and identify. In general, these can be divided into environmental hazards, physical hazards, and medical hazards. There may be substantial overlap between these categories.

Environmental hazards are those due to weather, terrain, altitude or depth, and wildlife including flora and fauna. These hazards can be addressed by careful planning with attention to adequate clothing, equipment, and logistics. Physical hazards include conditions which may cause physical harm to the traveler. Potentially dangerous activities such as rock-climbing, hang-gliding, and diving, as well as prevailing political and safety issues including war and loose firearms, crime, drugs, safety practices, and access to appropriate medical care are considered physical hazards. Some physical hazards are more difficult to address since some travelers deliberately place themselves in harm's way. Medical hazards include the risk of contracting infectious diseases such as typhoid, malaria, and dengue, as well as issues of food poisoning, unclean water, poor hygiene, risk of pulmonary embolism, and exacerbation of existing medical conditions. Taking into consideration all of these potential hazards in the chosen destination, plus one's own health status, emporiatric medicine aims to maintain the health and well-being of the traveler and minimize the risk of acquiring disease.

The Pre-Travel Medical Consultation

In order to minimize the possibility of travel related illness, the traveler must **gather as much information about the travel destination(s) and possible activities that he will engage in. A traveler should ideally** consult a medical practitioner with experience in travel medicine at least four weeks prior to departure. A longer preparation period may be needed if long-term travel or overseas work is expected, while consults as late as the day before travel may still be of benefit.

Itinerary

A detailed itinerary should be made available to the travel medicine provider prior to the consultation so that the practitioner can adequately determine possible medical, environmental, and physical risks to the traveler. This includes dates of departure and return, countries and cities which will be visited including accommodations, and whether the traveler will stay within urban limits or sojourn into rural and sylvan areas. Transit cities should also be included, as some countries have specific vaccination requirements for visas going to and from origin countries and entry may be denied on this basis.

An excellent reference that is used for risk assessment is the Centers for Disease Control and Prevention (CDC) Yellow Book, which has detailed descriptions of destinations and risks therein. Commercial travel medicine websites such as www.travax.com can be used to generate useful information, including patient handouts to help guide the traveler during his or her trip.

Clinic Visit

Basic demographic data along with specific health data is needed by the travel physician to make a complete risk assessment for the traveler. Aside from age, sex, and past medical history, a good vaccination history is also imperative in order to determine the need for boosters and

new vaccines. Allergies to food and drugs are elicited, as well as any reactions to previous vaccinations. Some vaccines may cause allergic reactions in those who are hypersensitive to poultry products because these may be produced in chicken or duck eggs. Influenza vaccines in particular may give a reaction in those allergic to poultry. A list of current medications is also useful to determine whether drug interactions may occur with those that may be prescribed for prophylaxis or treatment. An adequate supply of medication should be brought with the traveler since obtaining medication abroad may be difficult, along with the risk of counterfeit medication.

Physiologic states such as pregnancy, or breastfeeding status may present special problems during travel. For instance, access to birthing facilities abroad may need to be determined when traveling during late pregnancy. Certain airlines place restrictions on travel of pregnant women who are near term. Breastfeeding mothers may have to stop breastfeeding if certain prophylactic medicines are used, such as doxycycline and ciprofloxacin.

Interventions

Patient education on avoiding food- and water-borne diseases, as well as use of insect repellents such as N,N-diethyl-meta-toluamide (DEET)-containing preparations to avoid arthropod and other vector-borne diseases should be done. Instructions for self-medication for travel-related diarrhea (including antimicrobial use and oral rehydration solutions), as well as avoidance of contaminated water and ice should be emphasized. Use of sunblock and protective clothing should be mentioned, especially since some prophylactic medications such as doxycycline can cause photosensitivity. Special instructions regarding avoidance of specific illnesses (e.g., avoid wading in fresh water in schistosomiasis- and leptospirosis-endemic areas) should be given on a case-by-case basis. Risks of sexually transmitted diseases (STDs),

including HIV and AIDS, should be assessed and appropriate measures taken. Finally, arrangements should be made regarding access to medical treatment in emergent situations.

The need for any vaccinations and prophylaxis will depend on multiple factors. Most travel medicine authorities will make recommendations on the use of these interventions depending on risk of exposure, clinical impact, potential adverse reaction to the medication, and quarantine and infection risk to others. The only consistently required vaccine for travel purposes is yellow fever (for travel to endemic areas), while all others are recommended in varying degrees and depend on the type of exposures anticipated.

Vaccine-Preventable Diseases

Local recommendations for the immunization of adult Filipinos have been developed by the Philippine Society for Microbiology and Infectious Diseases with the Philippine Foundation for Vaccination in 2009 and are available online at www.pcp.org.ph. Indications for age, exposure, risk, and specific population are included, and travelers are urged to keep their immunizations up to date, whether or not international travel is planned. During pre-travel medical consultation, physicians have an excellent opportunity to review the immunization status of the traveler. Routine vaccinations are listed in Table 8.8 and are available in the country, while Table 8.9 shows vaccine-preventable diseases for selective use by travelers. In cases where there is uncertainty about vaccination status, serologic testing for antibody where available may be warranted depending on perceived risk of exposure.

Accelerated schedules are available for the travelers without adequate time prior to travel for routinely recommended travel immunizations. The U.S. Food and Drug Administration (FDA) has approved an accelerated dosing schedule that can afford protection against hepatitis A and hepatitis B,

Table 8.8. Vaccines for travelers

Category	Vaccine
1. Routine vaccination	Diphtheria, pertussis, and tetanus Hepatitis B Haemophilus influenzae type b Human papillomavirus Seasonal influenza* Measles, mumps and rubella Meningococcal disease** Pneumococcal disease Poliomyelitis Tuberculosis (BCG) Varicella
2. Selective use for travelers	Cholera Hepatitis A** Japanese encephalitis Rabies Tick-borne encephalitis Typhoid fever
3. Mandatory vaccination	Yellow fever** Meningococcal disease and polio (required by Saudi Arabia for pilgrims; updates are available from www.who.int/wer)
* Routine for certain age groups and risk factors, selective for general travelers **Included in the routine immunization program in several countries (Adapted from WHO International Travel and Health 2010)	

either as an individual or a combined vaccine in three weeks time, rather than the more lengthy standard 6-month dosing schedule. It should be noted though that while it is certainly better to have vaccination than not to have vaccination, accelerated schedules may offer only partial protection.

Travelers' Diarrhea

The most predictable and invariably the most common travel-related illness is travelers' diarrhea (TD), affecting 30 to 70% (up to 80% for high-risk destinations) of travelers depending on the destination and length of stay. Persons most affected are those traveling from an area of more highly developed standards of hygiene and sanitation to a less developed one. However, TD can still occur when traveling from a less developed to a more developed country

Table 8.9. Vaccines for selective use by travelers

Disease/ Etiologic agent	Transmission	Nature of disease	Occurrence	Risk for travelers	Preventive measures
Hepatitis A	Fecal-oral route	<ul style="list-style-type: none"> • Abrupt onset of fever, malaise, anorexia, nausea, and abdominal discomfort, followed within a few days by jaundice • Frequently acquired during early childhood and is usually asymptomatic or mild 	<ul style="list-style-type: none"> • Worldwide, levels of endemicity are related to hygienic and sanitary conditions in the geographic areas • Intermediate to high endemicity throughout the developing world 	<ul style="list-style-type: none"> • Risk varies with living conditions, length of stay, and the incidence of Hepatitis A infection in the area visited • Risk increases with visit to rural areas, trekking in back-country areas, or frequent eating or drinking in settings of poor sanitation 	<ul style="list-style-type: none"> • Two vaccine doses at 0 and 6-12 months (HAVRIX, VAXIA) • Accelerated schedule of the combined Hepatitis A + B vaccine (TWINRIX) – days 0, 7, and 21 PLUS a booster dose at 1 year • One dose administered at any time before departure can provide protection for most healthy persons, as early as 2 weeks after administration • Indication for Hepatitis A Immunoglobulin (passive vaccination) 0.02 mL/kg; <1yr old, allergy to a vaccine component; single dose provides protection up to 3 months
Hepatitis B	Transmission through blood or blood-derived fluids	<ul style="list-style-type: none"> • During acute infection: nausea, vomiting, abdominal pain, and jaundice; rashes, joint pain, and arthritis may occur 	<ul style="list-style-type: none"> • Low prevalence (<2%) in Northern and Western Europe, North America, Australia, New Zealand, Mexico, and South America • Intermediate (2-7%) in South, Central, and Southwest Asia, Israel, Japan, Eastern and Southern Europe, Russia, most areas surrounding the Amazon River basin, Honduras, and Guatemala • High ($\geq 8\%$) in Africa; Southeast Asia, China, Korea; the Middle East, except Israel; South and Western Pacific Islands; the interior Amazon River basin; and certain parts of the Caribbean (Haiti and the Dominican Republic) 	<ul style="list-style-type: none"> • Generally low, except for travelers to countries with high prevalence • Adventure travelers, Peace Corps volunteers, missionaries, and military personnel, may be at increased risk for infection 	<ul style="list-style-type: none"> • Vaccine given in a 3-dose series on a 0-, 1-, and 6-month schedule • Accelerated schedule for ENGERIX and TWINRIX as described above • Initiate vaccine, if indicated, even if it cannot be completed before departure

Disease/ Etiologic agent	Transmission	Nature of disease	Occurrence	Risk for travelers	Preventive measures
Typhoid and paratyphoid fever caused by <i>Salmonella</i> <i>enterica</i> <i>serotype</i> <i>typhi</i> , <i>S.</i> <i>paratyphi</i> A, B, or C	Fecal-oral route Transmission through sexual contact, especially among men who have sex with men, has been documented	<ul style="list-style-type: none"> Acute illness with fatigue, headache, relative bradycardia, anorexia, and fever that increases daily from low-grade to as high as 38.5-40°C Evanescant "rose spots" can occasionally be seen on the trunk 	<ul style="list-style-type: none"> South, East and Southeast Asia, Africa, the Caribbean, and Central and South America 	<ul style="list-style-type: none"> Risk is greatest for travelers to South Asia (6-30 times) higher than all other destinations; also at highest risk for quinolone- or multidrug-resistant strains 	<ul style="list-style-type: none"> Oral live-attenuated vaccine from Ty21a "strain" of <i>S. typhi</i>, OR a parenteral vaccine extracted from <i>S. typhi</i> strain capsule No protection against <i>S. paratyphi</i> typhoid Protects only 50-80% of recipients
Cholera caused by <i>Vibrio</i> <i>cholerae</i> bacteria, serogroups O1 and O139	Fecal-oral route Cholera affects only humans; there is no insect vector or animal reservoir host	<ul style="list-style-type: none"> An acute enteric disease varying in severity From asymptomatic to mild diarrhea to severe profuse watery diarrhea with nausea and vomiting and rapid development of dehydration In severe untreated cases, death may occur within a few hours due to circulatory collapse 	<ul style="list-style-type: none"> Cholera occurs mainly in poor countries with inadequate sanitation and lack of clean drinking water and in war-torn countries where the infrastructure may have broken down Developing countries: Africa and Asia, and to a lesser extent, those in Central and South America 	<ul style="list-style-type: none"> Low, provided that simple precautions are taken to avoid potentially contaminated food and water Humanitarian relief workers in disaster areas and refugee camps are at risk 	<ul style="list-style-type: none"> Inactivated <i>V. cholerae</i> strains in oral suspension available advised only for travelers going to areas with ongoing epidemics/ outbreaks
Japanese Encephalitis (JE) caused by Japanese encephalitis virus—a flavivirus	Bite of infected mosquitoes of the genus <i>Culex</i> ; Natural reservoirs: pigs and various wild birds	<ul style="list-style-type: none"> Range from asymptomatic to mild infections characterized by febrile headache or aseptic meningitis or encephalitis to severe (rapid onset and progression with headache, high fever and meningeal signs, permanent neurological sequelae) Approximately 25% of severe clinical cases have a fatal outcome 	<ul style="list-style-type: none"> JE is the leading cause of viral encephalitis in Asia Occurs in almost all of Asia Incidence declining in Japan and Korea due to immunization Increasing incidence in some regions of China, Bangladesh, India, Nepal, Pakistan, Northern Thailand, and Vietnam, due to flooding and related events 	<ul style="list-style-type: none"> Low, varies according to season, destination, duration of travel and activities Risk is higher in long-term travelers and expatriates Risk for travelers with extensive outdoor exposure (camping, hiking, bicycle tours, outdoor occupational activities, in particular in areas where flooding irrigation is practiced) 	<ul style="list-style-type: none"> Avoid mosquito bites Vaccine available but marketed outside the endemic countries

Disease/ Etiologic agent	Transmission	Nature of disease	Occurrence	Risk for travelers	Preventive measures
Tick-borne encephalitis caused by a flavivirus; Three subtypes: European subtype, the Far Eastern subtype, Siberian subtype	Bite of infected ticks; Ingestion of unpasteurized milk; No direct person- to-person transmission	<ul style="list-style-type: none"> Influenza-like illness, with a second phase of fever occurring in 10% of cases when encephalitis, paralysis, or death may develop Disease is seasonal; most cases occur during April to November 	<ul style="list-style-type: none"> The European subtype: Central and Eastern Europe, particularly Austria, Southern Germany, and Northern Switzerland; the Baltic states (Estonia, Latvia, Lithuania), the Czech Republic, Hungary, and Poland Far Eastern subtype: North-eastern Europe to China and Japan Siberian subtype: Northern Europe to Siberia 	<ul style="list-style-type: none"> Travelers are at risk when hiking or camping in rural or forested areas (up to an altitude of about 1,400 m) 	<ul style="list-style-type: none"> Avoid tick bites; if bitten, remove tick as soon as possible Two inactivated whole cell vaccines are available in Europe Outside endemic countries, the vaccines may not be licensed and will have to be obtained by special request
Yellow fever caused by yellow fever virus, an arbovirus, of the Flavivirus genus	Bite of infective <i>Aedes</i> and <i>Haemagogus</i> spp. mosquitoes in the forests of Africa and South America. Monkeys are the main reservoir of infection in forests; In Africa, mosquitoes infect both monkeys and humans, causing localized outbreaks	<ul style="list-style-type: none"> Asymptomatic Some lead to an acute febrile illness followed by a second febrile phase in 15% of patients Associated with musculoskeletal and abdominal pain 	<ul style="list-style-type: none"> Endemic in some tropical areas of Africa and central and South America Transmission can occur at altitudes up to 2,300 m in the Americas and possibly higher in Africa 	<ul style="list-style-type: none"> Risk in all areas where yellow fever is considered endemic, especially for visitors who enter forest and jungle area 	<ul style="list-style-type: none"> Avoid mosquito bites Yellow fever is the only disease for which the WHO requires an International Certificate of Vaccination for travelers The Philippines requires a vaccination certificate from all travelers over 1 year of age coming from endemic countries Filipinos traveling to endemic areas can get the vaccine and certificate from the Bureau of Quarantine, Port Area, Manila at telephone number (632) 527-4678. Vaccine is a live-attenuated virus 0.5 mL given subcutaneously as a single dose, booster doses given every 10 years

as a consequence of non-immunity to non-native enteric pathogens. Associated signs and symptoms include nausea, vomiting, abdominal cramps, and fever. Bacterial pathogens account for 80 to 90% of cases.

The most common pathogen causing TD is enterotoxigenic *Escherichia coli* (ETEC). Enteroadherent (EAEC) and other *E. coli* subtypes are also common pathogens in bacterial diarrhea. *Campylobacter jejuni*, *Shigella* spp., and *Salmonella* spp. are likewise usual pathogens but present with bloody diarrhea. Viruses, including norovirus, rotavirus, and astrovirus, have been isolated in 5 to 8% of TD. Protozoal pathogens, whose symptoms are slower to manifest and may be the cause of persistent diarrhea, collectively account for about 10% of diagnoses in longer-term travelers. *Giardia* is the major protozoan pathogen found in travelers. *Entamoeba histolytica* is relatively uncommon but can cause severe disease.

Diarrhea from toxins, colloquially known as “food poisoning,” involves the ingestion of preformed toxins in food, and present within 3 to 6 hours as vomiting and/or diarrhea that usually resolves spontaneously within 12 hours. Examples of the toxin mediated diarrhea include those caused by *Bacillus cereus* and *Staphylococcus aureus*. Some more exotic and potentially deadly toxins in food include neurotoxins from algal blooms (paralytic shellfish poisoning or red tide), ciguatera, and scombroid.

The importance of prevention can never be over-emphasized especially with regard to TD. For travelers to high-risk areas, education on food and beverage food choices is key to decreasing the risk of ingesting potential pathogens. These include avoiding undercooked and raw food, meticulous hand hygiene, exclusive use of bottled, boiled or filtered water (including water for tooth brushing), and eating only at hygienic and sanitary food establishments. Since a subset of travelers make it a point to eat indigenous cuisine from street vendors, these travelers should be educated

on the use of self-administered antibiotics. Prophylaxis using non-pharmacologic agents has been suggested, including the use of bismuth sulfate and probiotics, but the evidence for these interventions is still controversial. Prophylactic antibiotics are generally not recommended due to the possible emergence of resistance and potential side effects. However, short-term travelers who are high-risk hosts (e.g., immunosuppressed) or those taking critical trips during which diarrheal bouts could affect the purpose may be given prophylaxis. Attack rates of TD can be decreased from 40% down to 4% with prophylactic antibiotics but continuing changes in the patterns of resistance of the various choices of antibiotics should be considered. Fluoroquinolones have replaced cotrimoxazole and doxycycline as effective prophylactic agents. However, *Campylobacter* resistance to fluoroquinolone in Southeast Asian countries has prompted some authorities to use macrolides instead, especially for bloody diarrhea.

Most diarrheal diseases are self-limited and patients will recover in a few days. If warranted, as in bacterial TD, empiric treatment with an antibiotic directed at suspected bacterial pathogens are of benefit. Examples include single-dose or 1-day therapy with a fluoroquinolone, or azithromycin 500 mg/day for 1 to 2 days. More than antibiotics, however, it is very important for patients with diarrhea to replace volume losses. Fluid intake should be maintained with safe liquids. Special attention should be given to the use of ice in beverages, as ice may be made from unsafe water. If moderate to heavy diarrheal losses continue, oral rehydration salt (ORS) solution should be considered especially for children and the elderly.

Malaria

Malaria has been fully discussed in a previous chapter, but because it is preventable in travelers, prophylaxis will be briefly discussed

in this section. Malaria is found in over 100 countries, and greater than 125 million international travelers are at risk every year. It remains the most common cause of fever in returning travelers. Many travelers continue to acquire malaria, and more than 10,000 reported cases likely represent only the tip of the iceberg. Malaria, especially falciparum malaria can be a life-threatening disease, but is quite amenable to treatment when recognized early. In the Philippines, malaria risk exists throughout the year in areas below 600 m, except in the 22 provinces declared as malaria-free: Aklan, Albay, Benguet, Bilaran, Bohol, Camiguin, Capiz, Catanduanes, Cavite, Cebu, Guimaras, Iloilo, Northern Leyte, Southern Leyte, Marinduque, Masbate, Eastern Samar, Northern Samar, Western Samar, Siquijor, Sorsogon, Surigao Del Norte, and Metropolitan Manila. No risk is considered to exist in urban areas or in the

plains. *Plasmodium falciparum* accounts for 70 to 80% of cases, while *P. vivax* accounts for 20 to 30%. *P. falciparum* resistant to chloroquine and sulfadoxine-pyrimethamine has been reported, and so **chloroquine should not be taken to prevent malaria when traveling to endemic areas** (Table 8.10).

Anophelene mosquitoes that transmit malaria are known to be night biters. Preventing mosquito bites can be done through: wearing long-sleeved clothing and trousers, especially at night; use of insect repellents including DEET-containing and citronella-based preparations with periodic reapplication; and mosquito nets which should ideally be treated with insecticide. Garlic, vitamin B, and ultrasound devices do not prevent bites. Travelers should be wary of the symptoms of malaria especially fever occurring 1 week after the possible exposure and up to 2 years after the return.

Table 8.10. Recommended drugs used in the prophylaxis for malaria

Drug	Dose	Duration	Precautions
Atovaquone/proguanil (Malarone)	One adult tablet orally, daily (adult tablets contain 250 mg atovaquone and 100 mg proguanil hydrochloride)	Begin 1-2 days before travel to malarious areas; take daily at the same time each day while in the malarious area and for 7 days after leaving the area	<ul style="list-style-type: none"> Contraindications: severe renal impairment (creatinine clearance <30 mL/min), children <5 kg, pregnant women, and women breastfeeding infants Should be taken with food or a milky drink Not available in the Philippines
Doxycycline	100 mg orally, daily	Begin 1-2 days before travel to malarious areas; take daily at the same time each day while in the malarious area and for 4 weeks after leaving the area	<ul style="list-style-type: none"> Contraindicated in children <8 years of age and pregnant women
Mefloquine	228 mg base (250 mg salt) orally, once a week	Begin at least 2 weeks before travel to malarious areas; take weekly on the same day of the week while in the malarious area and for 4 weeks after leaving the area	<ul style="list-style-type: none"> Contraindications: allergy to mefloquine or related compounds (e.g., quinine, quinidine); active depression, a recent history of depression, generalized anxiety disorder, psychosis, schizophrenia, other major psychiatric disorders, seizures, cardiac conduction abnormalities

Specific Infectious Diseases Involving Potential Health Risks for Travelers

Specific infectious diseases that pose health risks to travelers have been listed and described in *International Travel and Health 2010* published by the World Health Organization (WHO). Inclusion in this list is based on a high enough prevalence in the country of travel to pose a significant risk to visitors (e.g., malaria, dengue), potentially fatal or severe morbidity resulting from exposure even if the agent is not very common (e.g., Ebola, rabies), and the potential for public health threat such as epidemics (e.g. avian influenza, HIV, influenza A/H1N1). The possibility of exposure is dependent on the presence of the infectious agents in the country of travel, while the risk of infection will depend on the itinerary, purpose of travel, and the traveler's behavior (Table 8.11).

Travel within the Philippines

Domestic destinations pose specific infectious and parasitic disease risks to the local traveler. Similar to international travel, food- and water-borne diseases still account for most travel-related illnesses locally, and so the same precautions for these diseases should be taken.

The Filipino traveler has likely been exposed to similar enteric pathogens in his place of origin so certain vaccinations such as that for hepatitis A may not be warranted. In those with co-morbid conditions such as HIV and hepatitis B, serologic testing for hepatitis A antibody may be warranted, and vaccination offered, if there is no immunity. Seroprevalence of Hepatitis B surface antigen (HBsAg) among Filipinos is high (>8%), and so vaccination is recommended for everyone, travelers and non-travelers alike. Typhoid fever is endemic in the country, occurring in all places all year round, causing a morbidity rate of 30.5/100,000 population, and a mortality rate of 1.7/100,000 population. Therefore, typhoid vaccination is advised.

Tuberculosis is highly prevalent in the Philippines, so no prophylaxis is needed for residents since everyone is considered exposed. Visitors from developed countries, who are not tuberculin test (PPD) positive, should have their status checked shortly after returning home to their native country. Influenza A (H1N1) is present, and has been reported in most of the urban destinations, and so vaccination, if available, should be done. Hand and general personal hygiene, as well as cough etiquette are recommended. All four dengue serotypes are present, and the country is considered hyperendemic for dengue, with transmission occurring throughout the year. According to the Department of Health National Epidemiology Center, an increase in the number of HIV cases has been reported in Metro Manila, Cebu, and Davao. Safe sexual practices and avoidance of risky behavior, especially in these areas, are advised. In Boracay Island in Aklan province, diarrhea caused by parasites and coliforms, skin diseases, and an increase in the cases of STDs have been reported. Malaria, food- and water-borne and skin diseases are the major concerns in traveling to Palawan. In Cagayan Valley, precautions for animal-borne diseases should be undertaken, most significantly, for leptospirosis. In addition, an outbreak of anthrax was recently reported. Infectious risks with travel to Davao include STDs, parasitism such as intestinal heterophyidiasis, leptospirosis, influenza, and dengue.

The Returned Traveler

Factors that influence the risk of illness during travel is similar to the pre-travel risks modified by the traveler's adherence to prescribed chemoprophylaxis and vaccination regimens (e.g., malaria prophylaxis), as well as activities during travel, and actual exposure to infectious agents during travel. Illnesses may begin during the travel period or may take weeks, months, or even years after return, to manifest, depending on the pathogen's

Table 8.1.1. Specific infectious diseases involving potential health risks for travelers

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
Amebiasis	<i>Previously discussed</i>					
Avian influenza	Highly pathogenic avian influenza A (H5N1) virus or other non-human influenza subtypes (e.g., H7, H9)	<ul style="list-style-type: none"> Contact with avian fecal material Bird-to-human, possibly environment-to-human and, very rarely, limited, non-sustained human-to-human transmission No evidence that properly cooked poultry or poultry products can be a source of infection 	<ul style="list-style-type: none"> Influenza-like illness, diarrhea, and other GI complaints Pneumonia with radiographic infiltrates of varying patterns Hemoptysis frequent Multi-organ failure, sepsis-like syndromes Fatality rate among hospitalized patients with H5N1 high (~60%) Severe illness also for H7N7 but mild for other avian influenza subtypes (e.g., H9N2) 	<ul style="list-style-type: none"> Only sporadic human infections have occurred to date Between November 2003 and July 2008, nearly 400 human cases of H5N1 were reported to WHO from 15 countries in Africa, South-East and Central Asia, Europe, and the Middle East 	<ul style="list-style-type: none"> Contact with environments such as live animal markets and poultry farms, any free-ranging or caged poultry, or surfaces that might be contaminated by poultry droppings increase risk 	<ul style="list-style-type: none"> Avoid consumption of undercooked eggs, poultry or poultry products Hand hygiene Avoid contact with animals and dead migratory birds Treatment and post-exposure prophylaxis: oseltamivir, zanamivir Although the vaccines are immunogenic, unknown effectiveness in preventing the H5N1 infection or reducing disease severity
Anthrax	<i>Bacillus anthracis</i>	<ul style="list-style-type: none"> Contact with products from infected animals (mainly cattle, goats, sheep), such as leather or woolen goods, or souvenirs made from animal skins Contact with soil containing anthrax spores 	<ul style="list-style-type: none"> Acute skin infection (most common form) Untreated infections may spread to regional lymph nodes and to the bloodstream, and may be fatal. 	<ul style="list-style-type: none"> Sporadic cases occur in animals worldwide Occasional outbreaks in Africa and Central Asia. 	<ul style="list-style-type: none"> Very low for most travelers 	<ul style="list-style-type: none"> No prophylaxis vaccine available for people at high risk because of occupational exposure to <i>B. anthracis</i> not commercially available in most countries Avoid direct contact with soil and with products of animal origin.
Brucellosis	Several species of <i>Brucella</i> bacteria	<ul style="list-style-type: none"> Direct contact with infected cattle (<i>Brucella abortus</i>), dogs (<i>B. canis</i>), pigs (<i>B. suis</i>), or sheep and Goats (<i>B. melitensis</i>) Consumption of unpasteurized (raw) milk or cheese 	<ul style="list-style-type: none"> Generalized infection with insidious onset, causing continuous or intermittent fever and malaise, which may last for months if not treated adequately Relapse common after treatment 	<ul style="list-style-type: none"> Worldwide, in animals Most common in developing countries, South America, Central Asia, the Mediterranean, and the Middle East 	<ul style="list-style-type: none"> Low Visit to rural and agricultural areas, intake of raw, unpasteurized milk increase the risk 	<ul style="list-style-type: none"> No prophylaxis Avoid consumption of unpasteurized milk and milk products Avoid direct contact with animals, particularly cattle, goats and sheep.

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
Chikungunya	Chikungunya virus—an alphavirus from the <i>Togaviridae</i> family	<ul style="list-style-type: none"> Bites of <i>Aedes aegypti</i> and <i>Aedes albopictus</i> mosquitoes → bite during daylight with peak activity in the early morning and late afternoon No direct person-to-person transmission 	<ul style="list-style-type: none"> Acute febrile illness with joint pains, particularly affecting the hands, wrists, ankles, and feet Recovery after a few days but joint pains may persist Muscle pain, headache, rash, leukopenia, GI, ocular, heart and neurologic complaints reported 	<ul style="list-style-type: none"> Chikungunya occurs in sub-Saharan Africa, South-East Asia and tropical areas of the Indian subcontinent, as well as islands in the South-Western Indian Ocean 	<ul style="list-style-type: none"> Risk for travelers in areas where Chikungunya is endemic 	<ul style="list-style-type: none"> No antivirals No vaccine Treatment is supportive. Mosquito bite precaution during both day and night
Coccidiomycosis	<i>Coccidioides</i> spp., a fungus	<ul style="list-style-type: none"> Inhalation of fungal conidia from dust 	<ul style="list-style-type: none"> Diseases range from asymptomatic to influenza-like illness to disseminated disease 	<ul style="list-style-type: none"> Mainly in the Americas 	<ul style="list-style-type: none"> Low Risk increases with activities that result in exposure to dust, e.g., dirt biking, excavation, construction 	<ul style="list-style-type: none"> No vaccine. Reduce exposure, wear well-fitted mask
Dengue	Dengue virus—a flavivirus with serotypes 1 to 4	<ul style="list-style-type: none"> Bite of <i>Aedes aegypti</i> mosquito during daytime No person-to-person transmission Monkey acts as reservoir host in west Africa and South-east Asia 	<ul style="list-style-type: none"> Occurs in 3 forms: (1) acute febrile illness followed by severe musculoskeletal pain and rash, (2) fever followed by thrombocytopenia and hemorrhagic complications, and (3) acute febrile illness followed by hypotension and shock 	<ul style="list-style-type: none"> Widespread in tropical and subtropical regions of Central and South America, South and South-East Asia, Africa, and Oceania The risk is lower at altitudes above 1,000 m 	<ul style="list-style-type: none"> Significant risk for travelers in areas where dengue is endemic 	<ul style="list-style-type: none"> No vaccine No antiviral Avoid mosquito bites.

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
<p>Giardiasis</p> <p>Hemorrhagic Fevers: Ebola and Marburg hemorrhagic fevers, Crimean-Congo hemorrhagic fever (CCHF), Rift Valley fever (RVF), Lassa fever</p>	<p><i>Previously discussed</i></p> <p>Ebola and Marburg belong to the Filoviridae family; CCHF and RVF belong to the Bunyaviridae family; Lassa fever belongs to the Arenaviridae family</p>	<ul style="list-style-type: none"> Transmitted by mosquitoes (RVF), ticks (CCHF), rodents (Lassa) or bats (Ebola, Marburg) For Ebola or Marburg viruses, infection from direct contact with the body fluids or secretions of infected patients, and less commonly, contact with tissues of diseased primates and other mammals Lassa fever virus transmitted through rodent excreta (via aerosols or direct contact) Blood/body fluid transmission for other hemorrhagic fevers Consumption of unpasteurized milk 	<ul style="list-style-type: none"> Severe acute viral infections, usually with sudden onset of fever, malaise, headache, and myalgia followed by pharyngitis, vomiting, diarrhea, skin rash, and bleeding Outcome is fatal in a high proportion of cases (more than 50%). 	<ul style="list-style-type: none"> Ebola and Marburg hemorrhagic fevers and Lassa fever occur in parts of sub-Saharan Africa. CCHF occurs in the steppe regions of Central Asia and in Central Europe, as well as in tropical and Southern Africa. RVF occurs in Africa and has recently spread to Saudi Arabia. Other viral hemorrhagic fevers occur in Central and South America. 	<ul style="list-style-type: none"> Low Travelers visiting rural or forest areas may be exposed. 	<ul style="list-style-type: none"> No prophylaxis Avoid mosquito bites. Avoid unpasteurized milk
<p>Hantavirus diseases</p> <p>Important examples are haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS)</p>	<p>Hantaviruses belong to the <i>Bunyaviridae</i> family</p>	<ul style="list-style-type: none"> Specific viruses carried by particular rodent hosts Direct contact with infected rodent feces, saliva or inhalation of virus via the excreta 	<ul style="list-style-type: none"> Acute viral diseases damaging vascular endothelium Increased vascular permeability hypotension, hemorrhagic manifestations, and shock Oliguria with HFRS; Respiratory failure caused by acute non-cardiogenic pulmonary edema occurs in HPS The outcome is fatal in up to 15% of HFRS cases and up to 50% of HPS cases. 	<ul style="list-style-type: none"> Worldwide, in rodents 	<ul style="list-style-type: none"> Low May increase in environment with many rodents and for adventure travelers, backpackers, campers 	<ul style="list-style-type: none"> No prophylaxis Avoid rodent exposure.

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
Hepatitis C	Hepatitis C virus, a hepatovirus	<ul style="list-style-type: none"> Parenteral transmission 	<ul style="list-style-type: none"> Gradual anorexia, abdominal discomfort, nausea and vomiting, followed by jaundice in some cases Most patients will develop a chronic infection, which may lead to cirrhosis and/or liver cancer. 	<ul style="list-style-type: none"> Worldwide, with regional differences in levels of prevalence 	<ul style="list-style-type: none"> Risk with unsafe behavior involving the use of contaminated needles for injection, acupuncture, piercing or tattooing, blood transfusion if the blood has not been screened for HCV Travelers engaged in humanitarian relief activities may be exposed to infected blood or other body fluids. 	<ul style="list-style-type: none"> No prophylaxis Safe sexual practices Blood and body-fluid precautions
Hepatitis E	Hepatitis E virus—not yet classified	<ul style="list-style-type: none"> Fecal-oral transmission Domestic animals as reservoir hosts, e.g., pigs 	<ul style="list-style-type: none"> Similar to Hepatitis A but more severe in pregnant women in their 3rd trimester 	<ul style="list-style-type: none"> Worldwide Most cases, both sporadic and epidemic occur in countries with poor standards of hygiene and sanitation. 	<ul style="list-style-type: none"> Risk when exposed to poor conditions of sanitation and drinking-water control 	<ul style="list-style-type: none"> No prophylaxis. Avoid potentially contaminated food and drinking-water.
Histoplasmosis	<i>Histoplasma capsulatum</i> , a dimorphic fungus	<ul style="list-style-type: none"> Inhalation of spores from soil contaminated with bat guano or bird droppings 	<ul style="list-style-type: none"> Most cases asymptomatic May cause acute pulmonary histoplasmosis (high fever, headache, cough, chills, weakness, pleuritic chest pain and fatigue) Most people recover spontaneously, but immunocompromised patients may develop severe disease. 	<ul style="list-style-type: none"> Worldwide 	<ul style="list-style-type: none"> Generally low Persons who visit endemic areas and are exposed to bird droppings and bat guano are at increased risk of infection. High-risk activities include spelunking, mining, and construction and excavation work. 	<ul style="list-style-type: none"> Avoid bat-inhabited caves. No vaccine available

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
Human Immunodeficiency virus (HIV) causing Acquired Immunodeficiency Syndrome (AIDS)	HIV-1 and HIV-2, which are retroviruses	<ul style="list-style-type: none"> • Transmission through blood and body fluids • Vertical transmission • Sexual transmission • Blood transfusion products 	<ul style="list-style-type: none"> • Acute retroviral syndrome similar with influenza-like illness • Wasting • Lymphadenopathy • Progressive decline in CD4+ count if without treatment • AIDS-defining illnesses once CD4+ significantly low 	<ul style="list-style-type: none"> • Worldwide, increased in areas such as Africa, prevalence differs in different risk groups (men having sex with men, IV drug users, commercial sex workers) 	<ul style="list-style-type: none"> • Unprotected sex and other high-risk behaviors 	<ul style="list-style-type: none"> • No vaccine yet • Post-exposure prophylaxis available • Highly-active antiretrovirals available for treatment • Safe sex
Influenza A (H1N1)	Influenza A(H1N1) virus – new reassortment virus not related to previous or current human seasonal influenza viruses	<ul style="list-style-type: none"> • Droplets expelled by coughing or sneezing • Direct contact with infected surfaces • No known instances of people being infected by exposure to pigs or other animals 	<ul style="list-style-type: none"> • Similar to seasonal influenza • Mild disease • High risk groups such as elderly or children may develop complications • Acute respiratory distress syndrome has also been seen in people with no known risk factors 	<ul style="list-style-type: none"> • Worldwide 	<ul style="list-style-type: none"> • Risk of acquiring influenza A (H1N1) now exists worldwide, especially in areas of overcrowding 	<ul style="list-style-type: none"> • Vaccine available • Cough etiquette • Hand hygiene • Self-isolation or quarantine if infected • Travelers should be aware of sign of severity and seek care quickly • Oseltamivir for prophylaxis and treatment available
Legionellosis	Various species of <i>Legionella</i> bacteria, frequently <i>Legionella pneumophila</i> , serogroup 1.	<ul style="list-style-type: none"> • Inhalation of contaminated water sprays or mists from air-conditioning cooling towers, hot-water systems, humidifiers, whirlpool spas and other water-containing devices • No direct person-to-person transmission 	<ul style="list-style-type: none"> • Two clinical forms: (1) Legionnaires' disease – acute pneumonia with anorexia, malaise, myalgia, headache, and rapidly rising fever → respiratory failure and death (2) Pontiac fever – influenza-like illness with spontaneous recovery after 2-5 days. 	<ul style="list-style-type: none"> • Worldwide 	<ul style="list-style-type: none"> • Generally low • Outbreaks occasionally occur in hotels and other facilities used by visitors • Risk factors: elderly, smokers, lung diseases, immunocompromised 	<ul style="list-style-type: none"> • No prophylaxis • Prevention of infection depends on regular cleaning and disinfection of possible sources • Treatment with azithromycin or fluoroquinolones

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
Leishmaniasis	Several species of the protozoan parasite <i>Leishmania</i>	<ul style="list-style-type: none"> Bite of female phlebotomine sandflies Reservoir hosts: dogs, rodents, and other mammals, including humans Blood/body fluid transmission also possible 	<ul style="list-style-type: none"> Three clinical forms: <ol style="list-style-type: none"> (1) Cutaneous – skin sores and chronic ulcers, generally self-limiting (2) Mucosal – caused by <i>Leishmania</i> species in Africa and the Americas which affect the nasal, oral and pharyngeal mucosal producing a disabling and mutilating disease (3) Visceral – affects the spleen, liver, bone marrow, and lymph nodes, producing fever, anemia → fatal if untreated 	<ul style="list-style-type: none"> Many countries in tropical and subtropical regions, including Africa, Central and South America, Asia, and the Mediterranean region More than 90% of cutaneous leishmaniasis occur in Afghanistan, Algeria, Brazil, Colombia, the Islamic Republic of Iran, Peru, Saudi Arabia, and Syria. More than 90% of mucosal leishmaniasis occur in Bolivia, Brazil, Ethiopia, and Peru. More than 90% of visceral leishmaniasis occur in Bangladesh, Brazil, Ethiopia, India, Nepal and Sudan 	<ul style="list-style-type: none"> Visitors to rural and forested areas in endemic countries are at risk. 	<ul style="list-style-type: none"> No prophylaxis Using insect repellents and insecticide-impregnated bednets Bite leaves a non-swollen red ring, which can alert the traveler to its origin.
Leptospirosis	Various spirochetes of the genus <i>Leptospira</i>	<ul style="list-style-type: none"> Direct or contact between the skin or mucous membranes and water, wet soil or vegetation contaminated by the urine of infected animals, notably rats 	<ul style="list-style-type: none"> Sudden onset of fever, headache, myalgia, chills, conjunctival suffusion, and skin rash May progress to meningitis, hemolytic anemia, jaundice, bleeding, and hepatorenal failure 	<ul style="list-style-type: none"> Worldwide, common in tropical countries 	<ul style="list-style-type: none"> Occupational risk for farmers engaged in paddy rice and sugar cane production Risk for visit to rural areas; contact with water in canals, lakes and rivers High-risk activities include canoeing, kayaking Outbreaks associated with eco-sports activities have occurred. 	<ul style="list-style-type: none"> Doxycycline 200 mg once a week until a week after possible exposure Avoid swimming or wading in potentially contaminated waters including canals, ponds, rivers, streams, and swamps. Avoid all direct or indirect contact with rodents.

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
Listeriosis	<i>Listeria monocytogenes</i>	<ul style="list-style-type: none"> Consumption of contaminated foods, particularly unpasteurized milk, soft cheeses, vegetables, and prepared meat products such as pâté Multiplicates readily in refrigerated contaminated food Vertical transmission 	<ul style="list-style-type: none"> May be mild except for high-risk population: newborn infants, pregnant women, elderly and immunocompromised Meningoencephalitis and/or septicemia in adults and newborn Fever, still birth, and abortion in pregnancy 	<ul style="list-style-type: none"> Worldwide, with sporadic incidence 	<ul style="list-style-type: none"> Generally low Risk is increased by consumption of unpasteurized milk and milk products, and prepared meat products. 	<ul style="list-style-type: none"> No prophylaxis Avoid consumption of unpasteurized milk and milk products. Pregnant women and immunocompromised individuals should take stringent precautions to avoid infection.
Lyme Diseases	<i>Borrelia burgdorferi</i> , a spirochete of several serotypes	<ul style="list-style-type: none"> Bite of infected ticks, both adults and nymphs, of the genus <i>Ixodes</i> Many species of mammals can be infected, and deer can act as an important reservoir. 	<ul style="list-style-type: none"> Usually has its onset in summer Early skin lesions have an expanding ring form, often with a central clear zone. Fever, chills, myalgia and headache are common. Meningeal, CNS complications, arthritis may occur weeks or months after the onset of illness. 	<ul style="list-style-type: none"> There are endemic foci of Lyme borreliosis in forested areas of Asia, Northwestern, Central and Eastern Europe, and the USA. 	<ul style="list-style-type: none"> Generally low Visitors to rural areas in endemic regions, particularly campers and hikers, are at risk. 	<ul style="list-style-type: none"> No prophylaxis Avoid tick-infested areas and exposure to ticks. If a bite occurs, remove the tick as soon as possible
Onchocerciasis	<i>Onchocerca volvulus</i> (nematode)	<ul style="list-style-type: none"> Through the bite of infected blackflies 	<ul style="list-style-type: none"> Chronic parasitic disease adult worms are found in fibrous nodules under the skin → discharge microfilariae, which migrate through the skin causing dermatitis, and reach the eye causing blindness 	<ul style="list-style-type: none"> Onchocerciasis occurs mainly in Western and Central Africa, also in Central and South America. 	<ul style="list-style-type: none"> Generally low, unless travel involves extensive exposure to the vectors in endemic areas 	<ul style="list-style-type: none"> No prophylaxis Avoid exposure to the bites of blackflies in endemic areas.

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
Parasitonyliasis	Previously discussed					
Plague	<i>Yersinia pestis</i>	<ul style="list-style-type: none"> Transmitted by fleas from rodents to other animals and to humans No direct person-to-person transmission does not occur except in the case of pneumonic plague → respiratory droplets 	<ul style="list-style-type: none"> Three clinical forms: <ol style="list-style-type: none"> Bubonic plague – from the bite of infected fleas → lymphadenitis, "buboes" Septicemia – dissemination in the blood results in meningitis, endotoxic shock and DIC → fatal Pneumonic plague – severe pneumonia → without prompt and effective treatment, 50-60% of cases fatal 	<ul style="list-style-type: none"> Wild rodent plague present in Central, Eastern and Southern Africa, South America, the Western part of North America, and in large areas of Asia. 	<ul style="list-style-type: none"> Generally low; travelers in rural areas of plague-endemic regions may be at risk, particularly if camping or hunting or if contact with rodents takes place 	<ul style="list-style-type: none"> A vaccine effective for high occupational exposure → not commercially available in most countries Treatment: tetracycline and fluoroquinolones. Avoid any contact with live or dead rodents.
Rabies	Rabies virus, a rhabdovirus of the genus <i>Lyssavirus</i>	<ul style="list-style-type: none"> Bite, penetrating scratch, licking of broken skin and mucosa of an infected animal Person-to-person transmission other than via organ transplant has not been laboratory-confirmed. 	<ul style="list-style-type: none"> Acute viral encephalomyelitis, which is fatal Initial signs include a sense of apprehension, headache, fever, malaise and sensory changes around the site of the animal bite. Excitability, hallucinations, aerophobia, hydrophobia (due to spasms of the swallowing muscles) 	<ul style="list-style-type: none"> Rabies is present in mammals in many countries worldwide Most rapid deaths occur in Africa and Asia. 	<ul style="list-style-type: none"> The risk to travelers in areas endemic for rabies is proportional to the probability of contact with potentially rabid mammals. 	<ul style="list-style-type: none"> No treatment Pre- and post-exposure prophylaxis available Modern cell-culture or embryonated egg vaccine given at Days 0, 3, 7 and 28 (post-exposure) Immunoglobulin for high-risk dog-bites available Local wound care

Disease	Cause	Transmission	Nature of disease	Geographic distribution	Risk for travelers	Prevention
SARS (Severe Acute Respiratory Syndrome)	SARS coronavirus (SARS-CoV) thought to be an animal virus from an as-yet -uncertain animal reservoir, perhaps bats, that spread to other animals (civet cats)	<ul style="list-style-type: none"> Transmission primarily from person-to-person occurring mainly during the second week of illness Few subsequent cases from laboratory accidents through animal-to-human transmission 	<ul style="list-style-type: none"> Flu-like illness, cough, and fever as the most frequently reported symptom Severe cases often evolve rapidly, progressing to respiratory distress. 	<ul style="list-style-type: none"> Guangdong, China – potential zone of re-emergence of SARS-CoV Other areas with human-to-human transmission that occurred from imported cases were Toronto, Hong Kong, Taiwan, Hanoi, and Singapore. 	<ul style="list-style-type: none"> Currently, no areas of the world are reporting transmission of SARS. During the height of the 2003 epidemic, the overall risk to travelers was low. 	<ul style="list-style-type: none"> No prophylaxis Experimental vaccines are under development. Follow any travel recommendations and health advice issued by WHO.
Schistosomiasis	<i>Previously discussed</i>					
Typanosomiasis	<i>Previously discussed</i>					
Typhus fever	<i>Rickettsia prowazekii</i>	<ul style="list-style-type: none"> Transmitted by the human body louse, infected by feeding on the blood of patients with acute typhus fever Infected lice excrete rickettsia onto the skin while feeding on a second host, who becomes infected by rubbing louse fecal matter or crushed lice into the bite wound. 	<ul style="list-style-type: none"> Headache, chills, high fever, prostration, coughing, and severe muscular pain followed After 5-6 days: dark spots on the trunk and to the rest of the body but usually NOT on the face, palms of the hands or soles of the feet Case-fatality rate is up to 40% without treatment. 	<ul style="list-style-type: none"> Louse-borne typhus fever is the only rickettsial disease that can cause explosive epidemics. Occurs in colder (i.e., mountainous) regions of Central and Eastern Africa, Central and South America, and Asia, Burundi, Ethiopia, and Rwanda. 	<ul style="list-style-type: none"> Very low for most travelers Humanitarian relief workers may be exposed in prisons, refugee camps, and other settings characterized by crowding and poor hygiene. 	<ul style="list-style-type: none"> No prophylaxis Cleanliness important in preventing infestation by body lice Insecticidal powders available for body-lice control and treatment of clothing for those at high risk of exposure

incubation period. According to the CDC, in terms of clinical severity, most travel-related illnesses are mild. Approximately 1 to 5% of travelers become sick enough to seek medical care either during or after travel. A careful travel history, therefore, should be part of the routine medical history for every ill patient, especially those with a febrile illness. Of particular concern are adventure travelers and persons visiting friends and relatives overseas, since they are at greater risk for becoming ill due to increased exposure to pathogens.

The most frequent health problems encountered by returned travelers are broken down as follows:

1. Persistent gastrointestinal illness (10%) – diarrhea was more common for travelers returning from South Central Asia
2. Skin lesions or rashes (8%) – insect bites, pyoderma, scabies, allergic rash, and cutaneous larva migrans; most frequent diagnoses among travelers returning from the Caribbean, Central or South America
3. Respiratory infections (5-13%), depending on the season of travel
4. Fever (up to 3%) – associated with the most serious complaints since certain conditions may be life-threatening (malaria) or may pose public health hazard (measles, tuberculosis); malaria, typhoid, and dengue were identified as the most frequent causes of systemic febrile illness among travelers from any region

While bacteria accounts for most TD cases, persistent symptoms suggest protozoan parasites as the etiology. In fact, in chronic diarrhea, parasites are commonly isolated, and

the likelihood of infection *vis-a-vis* an infection with a bacterial etiology increases with the duration of symptoms. Parasites may also be the likely etiologic agent for diarrhea unresponsive to antibacterials. Examples of intestinal parasites that may cause persistent symptoms include *Cryptosporidium parvum*, *Cystoisospora belli*, *Entamoeba histolytica*, *microsporidia*, and *Dientamoeba fragilis*, as well as *Cyclospora cayetanensis*. Other tests that may be requested in the evaluation of patients with persistent TD includes stool microscopy with at least three ova and parasite stool examinations, *Clostridium difficile* toxin assay, D-xylose test, duodenal aspirate, or empiric treatment for *Giardia*.

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Appendices

Bench Aids **for the** **diagnosis of** **intestinal** **parasites**



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Geneva

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Introduction

Identification of intestinal parasites

The goal of the microscopist in the diagnosis of intestinal parasitism is to ascertain the presence of parasites in faeces, whether they be minute protozoan cysts or large helminth eggs, and to identify them correctly. In some cases, the organisms are present in sufficient quantities to be found by direct examination of a small amount of faeces, i.e. the direct smear (see Plate 1). The addition of a drop of Lugol's iodine solution to the preparation will often bring out important morphological features of the parasites which will aid in their identification.

The identification of protozoan trophozoites and cysts in unstained faecal smears is a challenge even for the experienced microscopist and even under ideal conditions of collection and preparation of specimens. Trophozoites degenerate very rapidly so that faecal specimens must be examined promptly, permanent smears prepared for staining, or the specimen preserved in a special fixative such as merthiolate-formalin-iodine (MFI) as quickly as possible. Although direct examination of MFI-preserved material is useful, the microscopist must be experienced in the recognition of parasites in wet mounts.

Permanent-stained faecal smears are recommended for identification of protozoan parasites. Smears can be prepared from fresh faeces or from faecal material preserved in polyvinyl alcohol (PVA) or in sodium acetate-acetic acid-formalin (SAF). Other preservatives for faeces, such as 10% formalin, are not recommended for preparation of stained smears. The most commonly used permanent stains are trichrome and iron haematoxylin. Trichrome is easy to use and is especially good for smears made from fresh faeces or from PVA-preserved material; it is not recommended for use following SAF preservation. Iron haematoxylin is more difficult to use procedurally but gives excellent results on all types of faecal smears. In some instances, the use of more specialized staining techniques, such as acid-fast stains, will better demonstrate small coccidian organisms such as *Cryptosporidium* and *Cyclospora*. Even the minute spores of microsporidian species can be detected in faeces with modified or special staining procedures.

Most protozoan parasites are readily identified in permanent-stained smears. Even the most subtle and delicate features of these parasites can be visualized. As will be noted in the photomicrographs, the staining of organisms and faecal elements may vary considerably, even when the same stain is used. This may be due to many factors, including the age of the specimen when fixed, the fixative used, the thickness of the smears, and the time for destaining. We have attempted to provide the diagnostic features of all the common protozoan parasites using dichotomous keys and photomicrographs of all stages of each of the parasites as they appear unstained or stained in one or more of the stains described above.

In general, the diagnosis of intestinal helminths is less difficult than that of the intestinal protozoa. Helminth eggs are often easier to find and identify because of their size and their distinctive morphological features. While direct smears of fresh faeces will often demonstrate helminth eggs, it is usually more efficient for laboratories to do a simple concentration (see Plate 2) to avoid overlooking parasites that may be present in very small numbers. In some situations, such as large community-based surveys, specific objectives are limited to the detection of schistosome or soil-transmitted nematode (*Ascaris*, *Trichuris* and hookworm) infections. A modification of the direct smear procedure, the Kato-Katz technique (see Plate 3), is especially useful for field surveys for these infections because it also gives an estimation of the intensity of infection. We have provided images of the most common intestinal helminth parasites as they appear in faeces or, in some cases, in Kato-Katz preparations.

Finally, it is of the utmost importance that the microscopist is able to measure objects in the microscopic field. Accurate estimation of the size of organisms is important for correct diagnosis. Eyepiece reticules are available for virtually all microscopes. The reticule can be calibrated with the aid of a stage micrometer using the instructions provided overleaf.

Further reading

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Basic laboratory methods in medical parasitology. Geneva, World Health Organization, 1991.

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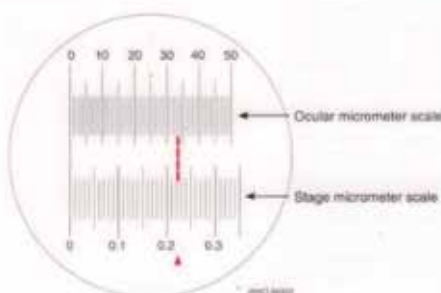


Introduction

Calibration of ocular micrometer

In order to measure elements in the microscopic field, it is necessary to have a measuring scale in the eyepiece of the microscope. Before it can be used, however, the scale must be calibrated. Ocular micrometers are flat glass discs on which a line scale divided into 50 or 100 small divisions has been etched. These divisions will have different measurement values depending on the power of the microscope objective used. The measurement values are calculated using a stage micrometer etched with a known calibrated scale of 0.1 mm divisions subdivided into 0.01 mm divisions. To calibrate the ocular micrometer, proceed as follows:

1. Remove the eyepiece (10X or other) from the microscope and unscrew the top or bottom lens, depending on its construction. Place the ocular scale on the diaphragm within the eyepiece with the etched surface on the undersurface of the reticule. Screw back the lens and re-insert the eyepiece into the microscope.
2. Place the stage micrometer on the microscope stage and focus the low-power objective on some portion of the scale with the 10X eyepiece.
3. Adjust the stage micrometer by moving the stage so that the 0 line of the ocular micrometer is exactly superimposed on the 0 line of the stage micrometer.



4. Without moving the stage micrometer, find another point at the extreme right where two other lines are exactly superimposed. This second set of superimposed lines should be as far to the right as possible from the 0 lines. This distance will vary with the objective used. At higher magnifications, the thickness of the etched lines may be so great that you need to look for superimposition of either the left or right edge of the individual lines.
5. Count the number of division lines on the ocular micrometer between the 0 line and the point where the second set of lines is superimposed. In the example provided in the figure, this number, indicated by the dotted line, equals 33 ocular units.
6. Then count the number of 0.1 mm division lines between the 0 line and the second superimposed line on the stage micrometer; in the figure, this number, indicated by the arrowhead, equals 0.22 mm.
7. To calculate the length represented by one ocular unit:

$$33 \text{ ocular units} = 0.22 \text{ mm}$$

$$1 \text{ ocular unit} = \frac{0.22 \text{ mm}}{33} = 0.0066 \text{ mm} = 6.6 \mu\text{m}$$

Thus, 1 ocular unit = 6.6 μm for this specific objective. Each objective on the microscope must be calibrated separately.
8. When all objectives have been calibrated prepare a simple chart that displays the calibration factor for each one.

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Plate 1 — Helminths

Note: All measuring bars = 25 µm



Normal fertile *Ascaris lumbricoides* eggs measure 35–75 µm by 35–50 µm, are golden yellow to brown in colour and are in the single-cell stage when passed in the faeces. The egg has conspicuous mammillations on its surface.



Typical fertile *Ascaris* egg as it appears in a Kato-Katz preparation.



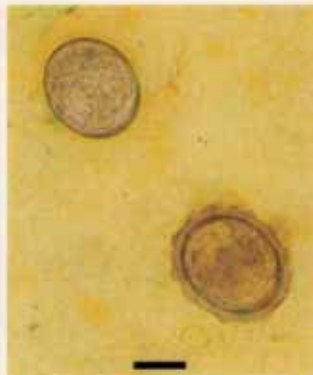
Typical infertile *Ascaris* eggs in faeces. These eggs are elongated and much larger in size (80–95 by 40–47 µm), have thin shells and a grossly irregular mammillated layer. The content of the egg is usually granular and lacks any organization.



Fertile (lower left) and infertile *Ascaris* eggs in a Kato-Katz preparation.



Ascaris. Sometimes, normal fertile eggs lack the mammillated layer and are referred to as "decoricated" eggs.



Ascaris. Normal and decoricated egg (upper left) in a Kato-Katz preparation.



Ascaris (upper) and *Trichuris* (lower) eggs in a Kato-Katz preparation.

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Plate 2

Faecal concentration procedure – formalin–ether/ethyl acetate/gasoline

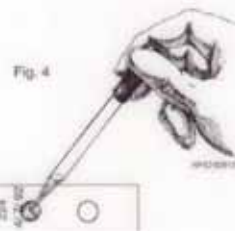
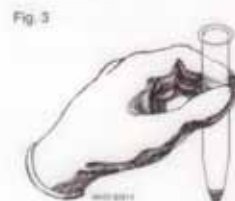
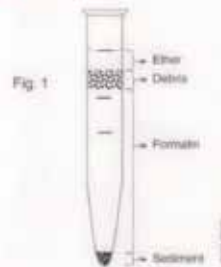
Materials and reagents

1. Centrifuge, with head and cups to hold 15 ml conical tubes. Sealed buckets must be used.
2. Centrifuge tubes, 15 ml, conical (make a graduation at 10 ml with a grease pencil).
3. Bottles, dispensing or plastic "squeeze", 250 or 500 ml.
4. Wooden applicator sticks, 1.45 x 2.0 mm.
5. Small beaker – 25, 50 or 100 ml.
6. 400 µm plastic or metal sieve or surgical gauze.
7. Microscope slides (75 x 25 mm).
8. Coverslips.
9. Pipettes, disposable Pasteur, with rubber bulbs.
10. Rubber stoppers for centrifuge tubes.
11. Rack or support for tubes.
12. Formalin, 10%^{*}.
13. Ether, ethyl acetate or, if these solvents are unavailable, gasoline. (**Caution:** Ether is highly volatile and will ignite and explode quickly if there is an open flame or spark nearby. Store open cans or bottles on an open shelf in the coolest part of the laboratory. Do not put opened containers of ether in a refrigerator as fumes escape, build up and may cause an explosion when the door is opened.)
14. Dropping bottles containing:
isotonic saline solution (0.85%, 8.5 g/l).
Lugol's iodine (1% solution).

^{*} For reagent preparation, consult the WHO publication, *Basic laboratory methods in medical parasitology*, 1991 (ISBN 92 4 154410 4).

Procedure

1. With an applicator stick add 1.0–1.5 g of faeces to 10 ml of formalin in a centrifuge tube and stir to form a suspension.
2. Strain the suspension through the 400 µm mesh sieve or 2 layers of wet surgical gauze directly into a different centrifuge tube or into a small beaker. Discard the gauze.
3. Add more 10% formalin to the suspension in the tube to bring the total volume to 10 ml.
4. Add 3.0 ml of ether (or ethyl acetate or gasoline) to the suspension in the tube and mix well by putting a rubber stopper in the tube and shaking vigorously for 10 seconds.
5. Remove the stopper, and place the tube in the centrifuge, balance the tubes and centrifuge at 400–500g for 2–3 minutes.
6. Remove the tube from the centrifuge; the contents consist of 4 layers: (a) top layer of ether (or ethyl acetate or gasoline), (b) a plug of fatty debris that is adherent to the wall of the tube, (c) a layer of formalin, and (d) sediment (Fig. 1).
7. Gently loosen the plug of debris with an applicator stick by a spiral movement and pour off the top 3 layers in a single movement, allowing the tube to drain inverted for at least five seconds. When done properly a small amount of residual fluid from the walls of the tube will flow back onto the sediment (Fig. 2, 3).
8. Mix the fluid with the sediment (sometimes it is necessary to add a drop of saline to have sufficient fluid to suspend the sediment) with a disposable glass pipette. Transfer a drop of the suspension to a slide for examination under a coverslip; an iodine-stained preparation can also be made (Fig. 4).
9. Examine the preparations with the 10X objective or, if needed for identification, higher power objectives of the microscope in a systematic manner so that the entire coverslip area is observed (see Plate 1, Fig. 4). When organisms or suspicious objects are seen, switch to higher magnification to see more detailed morphology of the object in question.



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Plate 2 – Helminths

Note: All measuring bars = 25 µm



Ascaris (upper), **Trichostrongyle** (middle) and **hookworm** (lower) eggs in the same microscopic field, illustrating their relative sizes.



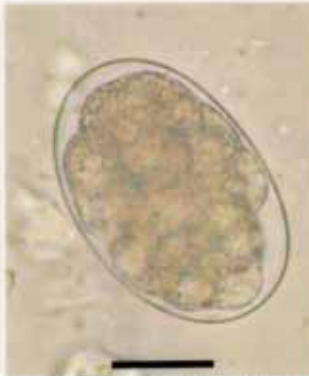
Typical **Trichostrongyle trichuris** eggs measure 50–55 µm by 22–24 µm, have a brown, smooth shell, bipolar prominences (plugs) and contain a single-cell ovum.



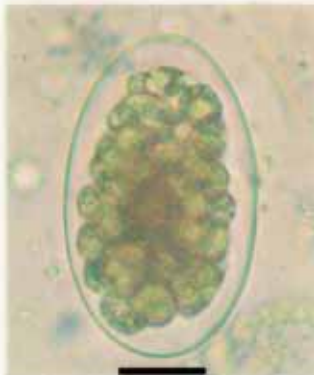
In a Kato-Katz preparation, **Trichostrongyle** eggs may appear larger and swollen with degenerated contents. The bipolar prominences and the layers of the shell are not sharply defined.



Hookworm eggs found in faeces are characteristically barrel-shaped with a thick, hyaline shell, they measure 60–75 µm by 36–40 µm. They are usually in the 4- or 8-cell stage in fresh faeces or in a more advanced stage of cleavage in faeces that have been kept at room temperature for over a few hours.



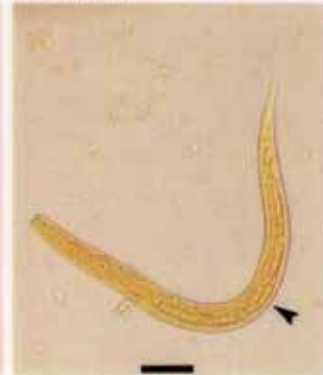
Hookworm eggs in Kato-Katz preparations are often almost round and the dividing ovum is increasingly difficult to see. In hot climates the glycerol will overclear the eggs and make them invisible 30–60 minutes after preparation.



Trichostrongyle eggs resemble hookworm eggs but are larger (75–95 µm by 40–50 µm) and more elongated in shape. The ovum is in an advanced stage of cleavage when passed in the faeces.



Trichostrongyle is another strongyle parasite which infects humans, mostly in southern Africa. The egg resembles the hookworm egg and measures about 85x52 µm. It tends to be in an advanced stage of cleavage when passed in the faeces.



Strongyloides stercoralis infection is routinely diagnosed by the presence in faeces of first stage rhabditoid larvae of 180–300 µm by 14–20 µm. Larvae have a short buccal capsule, an attenuated tail and a prominent genital primordium (arrow).

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Plate 3

Kato-Katz technique – cellophane faecal thick smear

Materials and reagents

1. Applicator sticks, wooden.
2. Screen, stainless steel, nylon or plastic; 60–105 mesh (Fig. 1).
3. Template, stainless steel, plastic, or cardboard (Fig. 1). Templates of different sizes have been produced in different countries. A hole of 9 mm on a 1 mm thick template will deliver 50 mg of faeces; a hole of 6 mm on a 1.5 mm thick template, 41.7 mg; and a hole of 6.5 mm on a 0.5 mm thick template, 20 mg. The templates should be standardized in the country and the same size of templates should always be used to ensure repeatability and comparability of prevalence and intensity data.
4. Spatula, plastic (Fig. 1).
5. Microscope slides (75 x 25 mm).
6. Hydrophilic cellophane, 40–50 µm thick, strips 25 x 30 or 25 x 35 mm in size (Fig. 2).
7. Flat-bottom jar with lid (Fig. 2).
8. Forceps.
9. Toilet paper or absorbent tissue.
10. Newspaper.
11. Glycerol-malachite green or glycerol-methylene blue solution (1 ml of 3% aqueous malachite green or 3% methylene blue is added to 100 ml of glycerol and 100 ml of distilled water and mixed well). This solution is poured onto the cellophane strips in a jar and left for at least 24 h prior to use.

Procedure

1. Place a small mound of faecal material on newspaper or scrap paper and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top (Fig. 3).
2. Scrape the flat-sided spatula across the upper surface of the screen to collect the sieved faeces (Fig. 4).
3. Place template with hole on the centre of a microscope slide and add faeces from the spatula so that the hole is completely filled (Fig. 5). Using the side of the spatula pass over the template to remove excess faeces from the edge of the hole (the spatula and screen may be discarded or, if carefully washed, may be reused).
4. Remove the template carefully so that the cylinder of faeces is left on the slide.
5. Cover the faecal material with the pre-soaked cellophane strip (Fig. 6). The strip must be very wet if the faeces are dry and less so if the faeces are soft (if excess glycerol solution is present on upper surface of cellophane wipe with toilet paper). In dry climates excess glycerol will retard but not prevent drying.
6. Invert the microscope slide and firmly press the faecal sample against the hydrophilic cellophane strip on another microscope slide or on a smooth hard surface such as a piece of tile or a flat stone. The faecal material will be spread evenly between the microscope slide and the cellophane strip (Fig. 7). It should be possible to read newspaper print through the smear after clarification (Fig. 8).
7. Carefully remove slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the bench with the cellophane upwards. Water evaporates while glycerol clears the faeces.
8. For all except hookworm eggs, keep slide for one or more hours at ambient temperature to clear the faecal material prior to examination under the microscope. To speed up clearing and examination, the slide can be placed in a 40 °C incubator or kept in direct sunlight for several minutes.
9. *Ascaris* and *Trichuris* eggs will remain visible and recognizable for many months in these preparations. Hookworm eggs clear rapidly and will no longer be visible after 30–60 minutes. Schistosoma eggs may be recognizable for up to several months but it is preferable in a schistosomiasis endemic area to examine the slide preparations within 24 hours.
10. The smear should be examined in a systematic manner (see Plate 1, Fig. 4) and the number of eggs of each species reported. Later multiply by the appropriate number to give the number of eggs per gram of faeces (by 20 if using a 50 mg template; by 50 for a 20 mg template, and by 24 for a 41.7 mg template). With high egg counts, to maintain a rigorous approach while reducing reading time, the Stoll quantitative dilution technique with 0.1 mol/litre NaOH may be recommended (see Basic laboratory methods in medical parasitology, WHO, 1991).



Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7



Fig. 8

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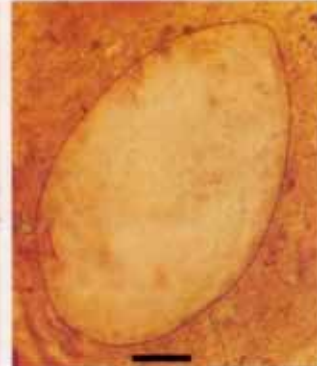


Plate 3 — Helminths

Note: All measuring bars = 25 µm



Schistosoma mansoni eggs are large, measuring 114–175 µm by 45–70 µm, have a thin, transparent shell and a prominent lateral spine, and contain a miracidium. If the spine is hidden from view, gently tapping the cover slip may expose it.



S. mansoni in Kato-Katz preparations are easily identified on the basis of size, shape and presence of the lateral spine.



Schistosoma japonicum eggs are smaller than those of *S. mansoni* and *S. haematobium*. They measure 70–100 µm by 35–60 µm and tend to be round to oval in shape, have a thin shell and a small, inconspicuous, lateral spine. The eggs contain a miracidium. Frequently, faecal debris adhered to the egg surface or orientation may obscure the spine.



In Kato-Katz preparations, the spine of the egg of *S. japonicum* is rarely seen and the miracidium quickly becomes inequipped. Size and thin shell help identify the species.



The eggs of *S. haematobium* have a terminal spine and contain a miracidium. They measure 110–170 µm by 50–70 µm. These eggs are usually found in the urine but occasionally they may also be found in faeces.



The eggs of *Schistosoma intercalatum* are usually larger than those of *S. haematobium*, measure about 140–240 µm, are typically found in faeces and have an equatorial bulge.

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Plate 4 – Helminths

Note: All measuring bars = 25 µm



Clonorchis sinensis eggs are 27–35 µm by 12–18 µm, have a veiled operculum and usually a small protuberance at the apical end. The shell may have minute adherent debris. Eggs in faeces contain a miracidium. ***Dyctiocheila*** eggs are similar.



Metagonimus yokogawai eggs measure 20–30 µm by 12–17 µm, have an inconspicuous operculum and lack a knob or protuberance at the abopercular end; the shell is usually devoid of adherent debris. Eggs in faeces contain a miracidium.



Fasciola hepatica eggs are usually 130–150 µm by 60–90 µm, have an inconspicuous operculum, are unembryonated, and often have a shell irregularity at the abopercular end (the latter is not seen in the similar ***Fasciolopsis buski*** egg).



Paragonimus westermani eggs usually measure 90–120 µm by 45–70 µm, are golden brown in colour, thick shelled, unembryonated in faeces or in sputum and have a prominent operculum. The shell is thickened at the abopercular end.



Paragonimus strobilatoralis eggs, an African species, are usually smaller than those of ***P. westermani***, i.e. 50–95 µm by 30–55 µm, and the operculum is less prominent.



Dipyllobothrium latum. These operculate cestode eggs usually measure 58–75 µm by 40–50 µm, are unembryonated in faeces, and may have a knob or small protuberance at the abopercular end.



Taenia spp. eggs are all identical, i.e. 31–43 µm in diameter, with a thick, prismatic-appearing shell wall, and contain a 6-hooked embryo, the oncosphere. Occasionally a thin, hyaline primary embryonic membrane may be retained around these eggs.



Hymenolepis diminuta eggs measure 70–85 µm long by 60–80 µm wide, are spherical, yellowish-brown, and contain a 6-hooked embryo. There are no filaments, as in ***H. nana***.



Hymenolepis nana eggs are usually spherical, 30–47 µm in diameter, have a thin hyaline shell and contain a 6-hooked oncosphere. There are two polar thickening on the membrane around the oncosphere from which arise 4–8 filaments extending into the space between the oncosphere and the outer shell.

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Plate 5

Staining procedures for protozoa in faeces

The use of Lugol's iodine for staining wet mount preparations from fresh or formalin-preserved faecal specimens is described on Plate 1. Here are presented some procedures for permanent staining of smears prepared from fresh, PVA- or SAF-preserved faecal material. Many details of preparation of faecal smears and the application of various staining procedures are also presented in the references listed in the Introduction.

Permanent stains for faecal smears

A. Trichrome stain

Use. Very good stain for fresh and PVA-preserved faecal smears; does not give good staining results with SAF preservation.

Preparation. Add 10 ml of glacial acetic acid to 5 g of chromotrope 2R, 3 g of light green SF and 7 g of phosphotungstic acid in a clean flask. Swirl to mix and let stand for 30 min. Add 1000 ml of distilled water and mix thoroughly; the stain should be a deep purple. Store in a glass-stoppered bottle; the stain is stable and is used undiluted.

Staining procedure. Place slides, fixed in either Schaudinn's fixative or PVA, into 70% alcohol for 2 min. Add Lugol's diluted iodine solution to 70% ethanol to produce a colour of strong tea; place slides in the solution for 5 min. Place slides in two changes of 70% alcohol. Stain slides in undiluted trichrome stain for 10 min. Remove slides, drain thoroughly, and place them in 100% acidified alcohol (prepared by adding 4.5 ml of glacial acetic acid to 1 litre of 90% ethanol) for 2–3 seconds. Dip slides in 95% alcohol to rinse and then dehydrate through 100% ethanol and xylene or through carbol-xylene mixture. Using resinous mounting medium, place a coverslip on the smear.

B. Iron haematoxylin stain

Use. Very good stain for fresh, PVA- or SAF-preserved faecal smears.

Preparation.

Stock solution A: dissolve 1 g of haematoxylin crystals in 100 ml of 95% alcohol; allow solution to stand in light for 1 week and then filter.

Stock solution B: mix 1 g of ferrous ammonium sulfate, 1 g of ferric ammonium sulfate and 1 ml of hydrochloric acid in 97 ml of distilled water.

Prepare a working solution by combining 25 ml each of stock solutions A and B; prepare at least 3–4 h prior to staining. Prepare picric acid solution for destaining by adding 25 ml of saturated aqueous picric acid to 25 ml of distilled water.

Staining procedure. Place slides into 70% alcohol for 5 min; into 50% alcohol for 2 min; into tapwater for 5 min; into working haematoxylin stain solution for 10 min; into distilled water for 1 min; into picric acid solution for 1 min; into running tapwater for 10 min; into 70% alcohol containing 1 drop of ammonia for 5 min; and into 95% alcohol for 5 min. Dehydrate through 100% ethanol and xylene or through carbol-xylene mixture. Using resinous mounting medium, place a coverslip on the smear.

C. Modified Ziehl-Neelsen technique (acid-fast stain)

Use. For detection of *Cryptosporidium*, *Cyclospora*, and other coccidian infections.

Reagents. Carbol-fuchsin, formalin, HCl-ethanol solution, glycerol-malachite green (or methylene blue) solution, HCl-methanol solution. For preparation of reagents see the WHO publication *Basic laboratory methods in medical parasitology*, 1991.

Staining procedure. Prepare a thin smear of faeces; air-dry and fix in methanol for 2–3 min. Stain with cold carbol-fuchsin for 5–10 min. Differentiate in 1% HCl-ethanol until colour ceases to flow out of smear. Rinse in tapwater. Counterstain with 0.25% malachite green (or methylene blue) for 30 sec. Rinse in tapwater. Blot or drain dry.

Bench Aids for the Diagnosis of Intestinal Parasites

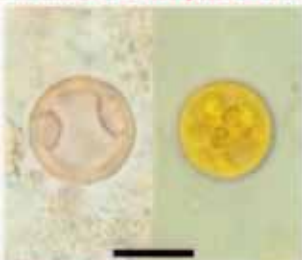
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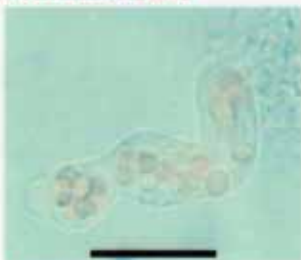
Plate 5 — Protozoa

Note: All measuring bars = 10 µm

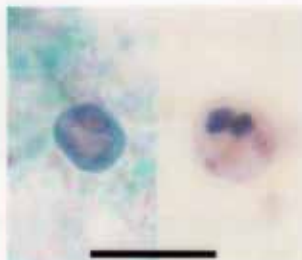
Entamoeba histolytica and Entamoeba hartmanni



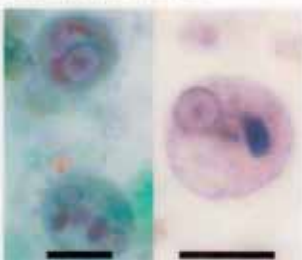
Left: *E. histolytica* trophozoite cyst in MB wet mount, large glycogen vacuole lies between nuclei. Right: *E. histolytica* mature cyst in cystine wet mount, 3 of the 4 nuclei are seen.



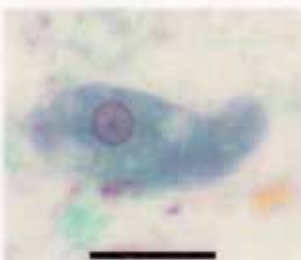
E. histolytica living trophozoite containing many red blood cells, unstained wet mount.



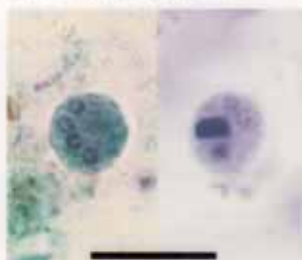
E. hartmanni trophozoites. Left: glycogen vacuole and chromatin bodies present, trichrome. Right: chromatin bodies present, iron haematoxylin.



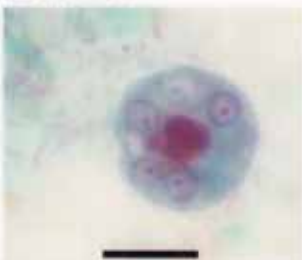
Left: *E. histolytica* trophozoite (left) and trophozoite cyst, each with glycogen vacuole and chromatin bodies, trichrome. Right: *E. histolytica* trophozoite cyst with chromatin bodies present, iron haematoxylin.



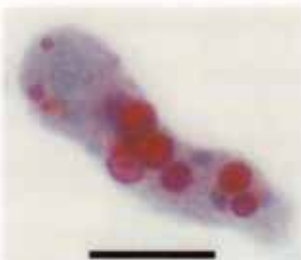
E. histolytica trophozoite, trichrome.



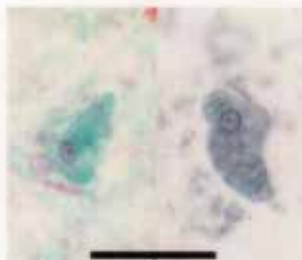
Left: *E. hartmanni* trophozoite with 4 nuclei, trichrome. Right: trophozoite cyst with 1 nucleus in sharp focus and chromatin bodies present, iron haematoxylin.



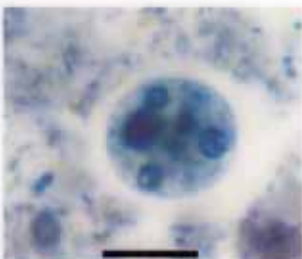
E. histolytica mature cyst with 4 nuclei and chromatin bodies, trichrome.



E. histolytica trophozoite with ingested, red-staining erythrocytes, nucleus visible along lower margin of organism, trichrome.



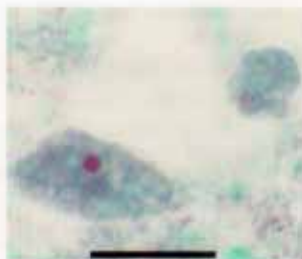
E. hartmanni trophozoites. Left trichrome. Right iron haematoxylin.



E. histolytica mature cyst showing 3 of the 4 nuclei and chromatin bodies which are not in focus, iron haematoxylin.



E. histolytica trophozoite, iron haematoxylin.



E. hartmanni (right) and *Isodamoeba butschlii* (left) trophozoites, trichrome. Note size difference.

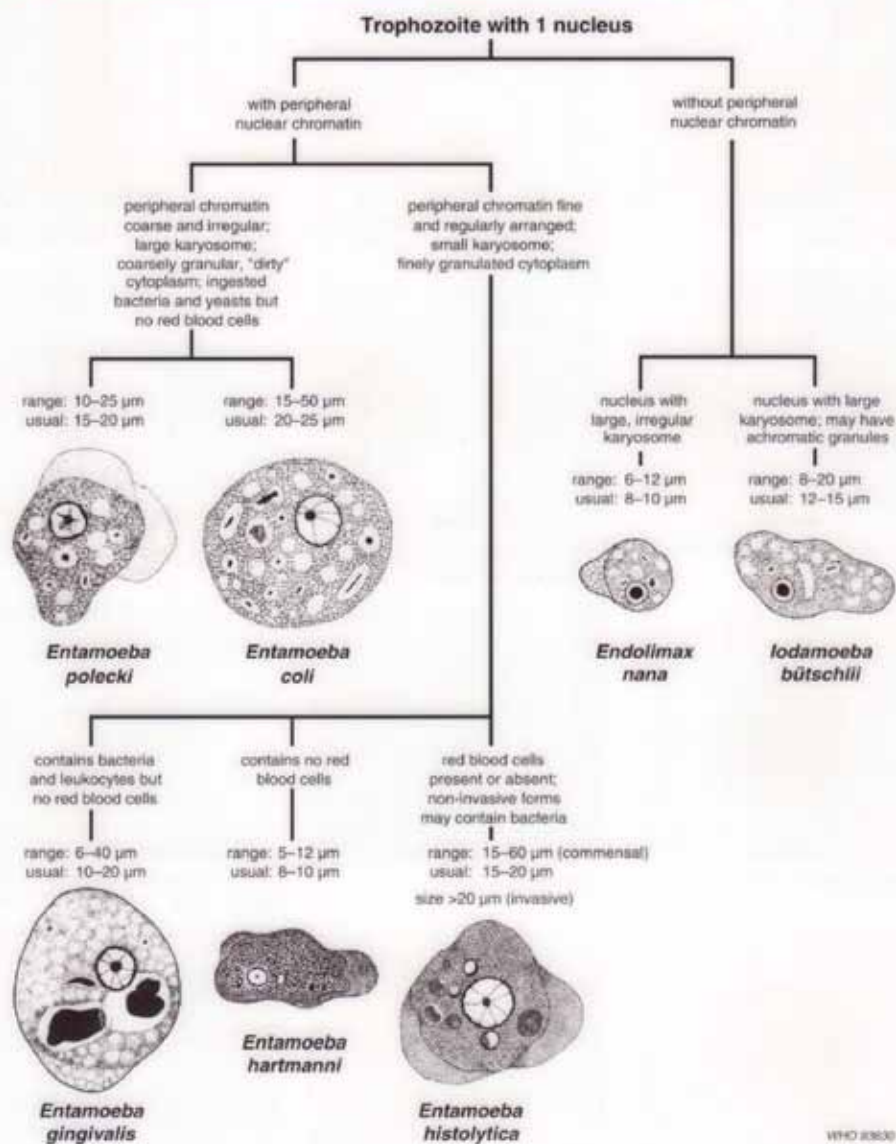
Bench Aids for the Diagnosis of Intestinal Parasites

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Plate 6

Key for the identification of amoebic trophozoites in stained smears



WHO 33530

Bench Aids for the Diagnosis of Intestinal Parasites

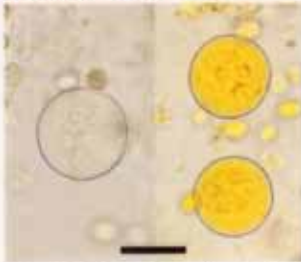
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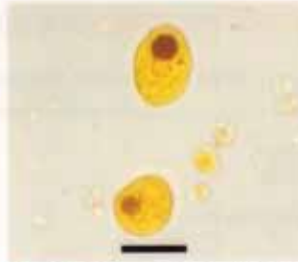
Plate 6 – Protozoa

Note: All measuring bars = 50 µm

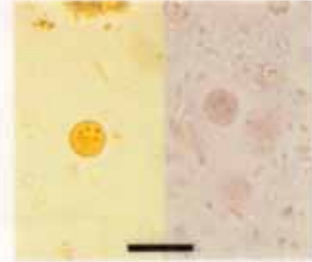
Commensal amoebae



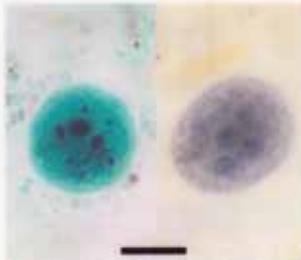
Entamoeba coli mature cysts. Left: unshaded formalin wet mount. Right: iodine-stained wet mount.



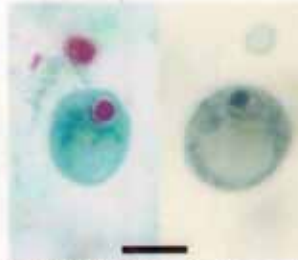
Ictamoeba biluchii cysts in iodine wet mount. Note brown-staining glycogen vacuoles in each. The nucleus is typically not visible in such preparations.



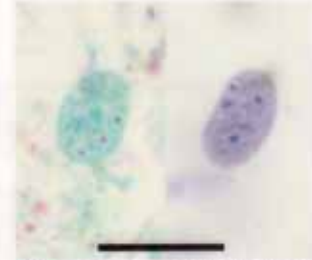
Entelima nana cysts in wet mounts. Left: a cyst, stained in iodine, shows 5 of the 4 nuclei. Right: 3 cysts in BMF, with the top one showing 3 of the 4 nuclei.



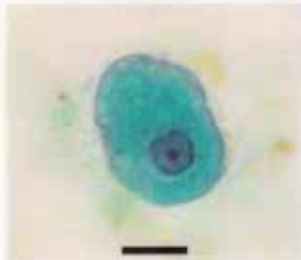
E. coli trypomastotes stained in trichrome (left) and iron haematoxylin (right).



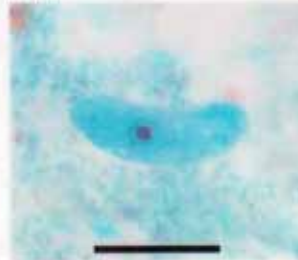
Cysts of *I. biluchii*. On the left, stained with trichrome, vacuole is not as clearly seen as it is on the right, stained with iron haematoxylin, with these stains the single nucleus with the large karyosome is readily seen.



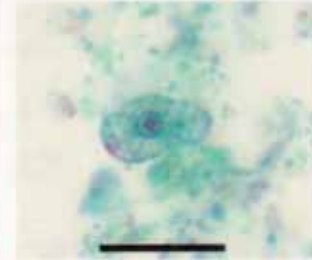
E. nana cysts. Left: 3 of 4 nuclei visible, trichrome. Right: all 4 nuclei seen, iron haematoxylin.



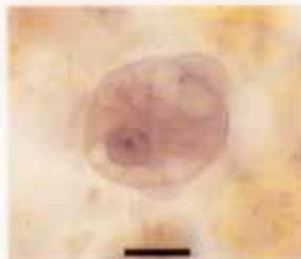
E. coli trypomastote, trichrome. Note irregular peripheral chromatin on nuclear membrane.



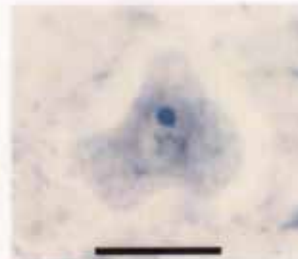
I. biluchii trypomastote, trichrome.



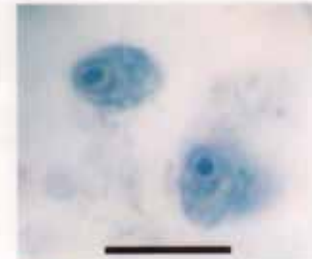
E. nana trypomastote, trichrome. Small size of organism and the large karyosome nearly filling the nucleus which lacks peripheral chromatin are diagnostic.



E. coli trypomastote, iron haematoxylin. Note large, off-centre karyosome in nucleus.



I. biluchii trypomastote, iron haematoxylin.



E. nana trypomastotes, iron haematoxylin.

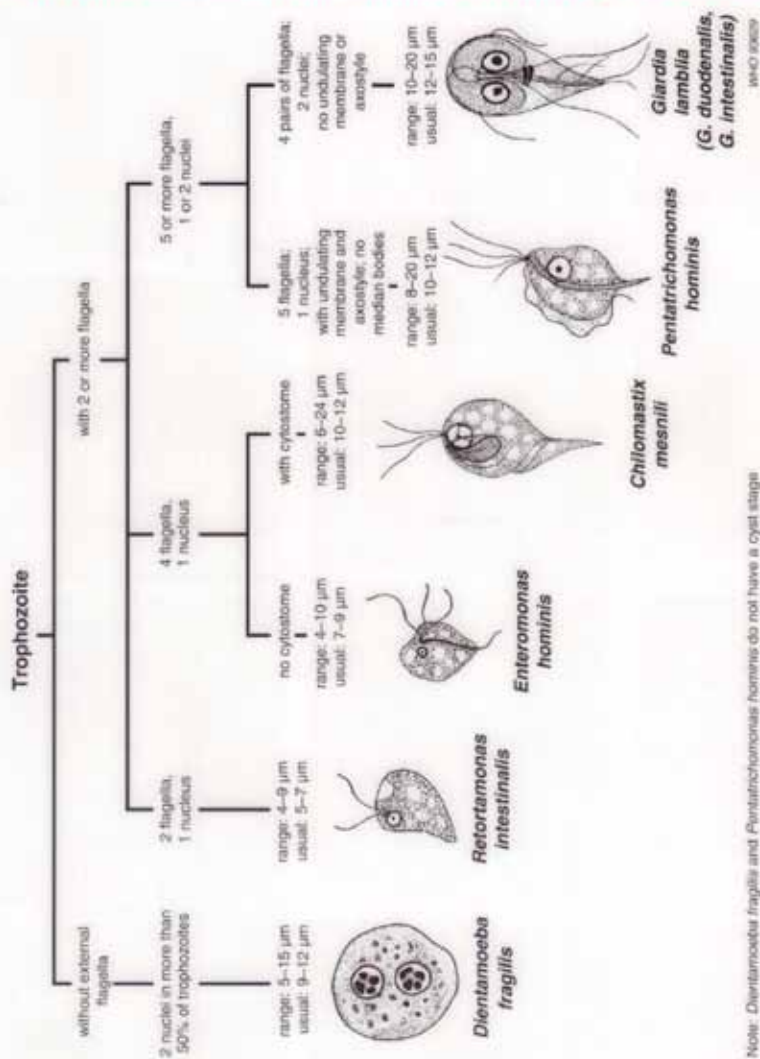
Bench Aids for the Diagnosis of Intestinal Parasites

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Plate 7

Key for the identification of trophozoites of intestinal flagellates in stained smears



Bench Aids for the Diagnosis of Intestinal Parasites

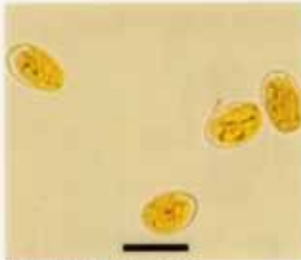
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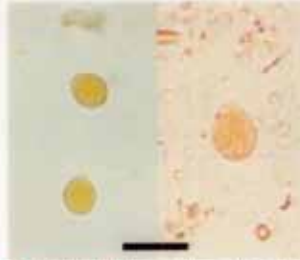
Plate 7 — Protozoa

Note: All measuring bars = 10 µm

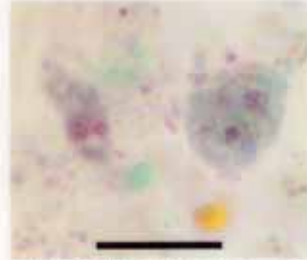
Intestinal flagellates



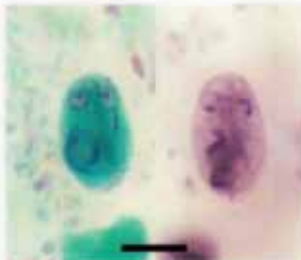
Giardia lamblia cysts, iodine wet mount.



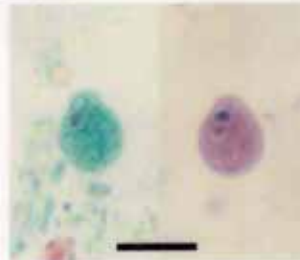
Chilomastix mesnili cysts, iodine wet mounts. Two cysts at left are at lower magnification and show typical lemon-shaped appearance; at higher magnification (right), nucleus and cytostome are fairly visible.



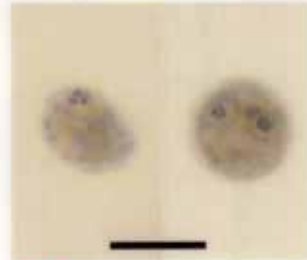
Dientamoeba fragilis trypomastotes, trichrome. On the left, only one of two nuclei is clearly seen. With trichrome it is characteristic for trypomastotes to take a pale stain. No cyst stage occurs in this species.



D. lamblia cysts stained in trichrome (left) and iron-haematoxylin (right).



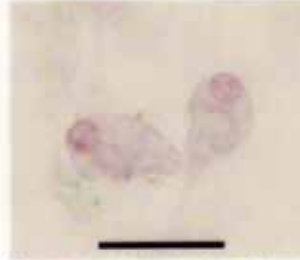
C. mesnili cysts in trichrome (left) and iron-haematoxylin (right). Both show typical lemon-shaped appearance and in the figure on the right the cytostome is fairly visible.



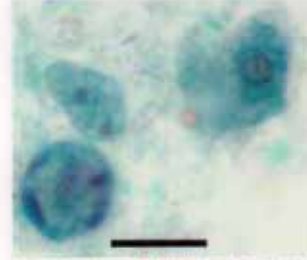
D. fragilis trypomastotes, iron-haematoxylin. Left: a unilocular form with karyosome fragmented into 3 pieces. Right: two nuclei, both showing fragmentation of the karyosomes, are present.



D. lamblia trypomastotes, trichrome.



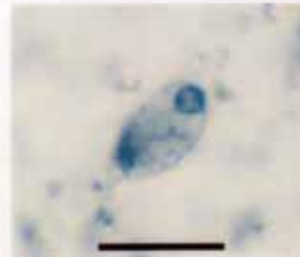
C. mesnili trypomastotes, trichrome. With trichrome trypomastotes often take a pale stain as seen here; one organism shows finely pointed posterior end and fairly staining cytostome.



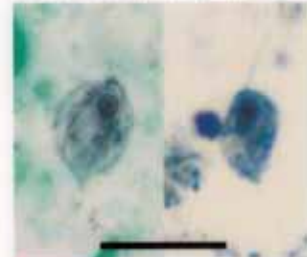
A binucleate trypomastote of *D. fragilis* which is more delicately stained, is seen between an *E. histolytica* trypomastote (upper right) and a smaller, uninucleate cyst of *E. histolytica* containing chromatin bodies. Note size differences. Trichrome.



D. lamblia trypomastotes, iron-haematoxylin. Three are ventral views of the organisms and two are seen in lateral aspect.



C. mesnili trypomastotes, iron-haematoxylin. Note nucleus at anterior end and finely pointed posterior end.



Pentastikomonas hominis trypomastotes stained in trichrome (left) and iron-haematoxylin (right). Note nucleus and axostyle in organism on left, and anteriorly directed, fairly staining flagellum in organism on right.

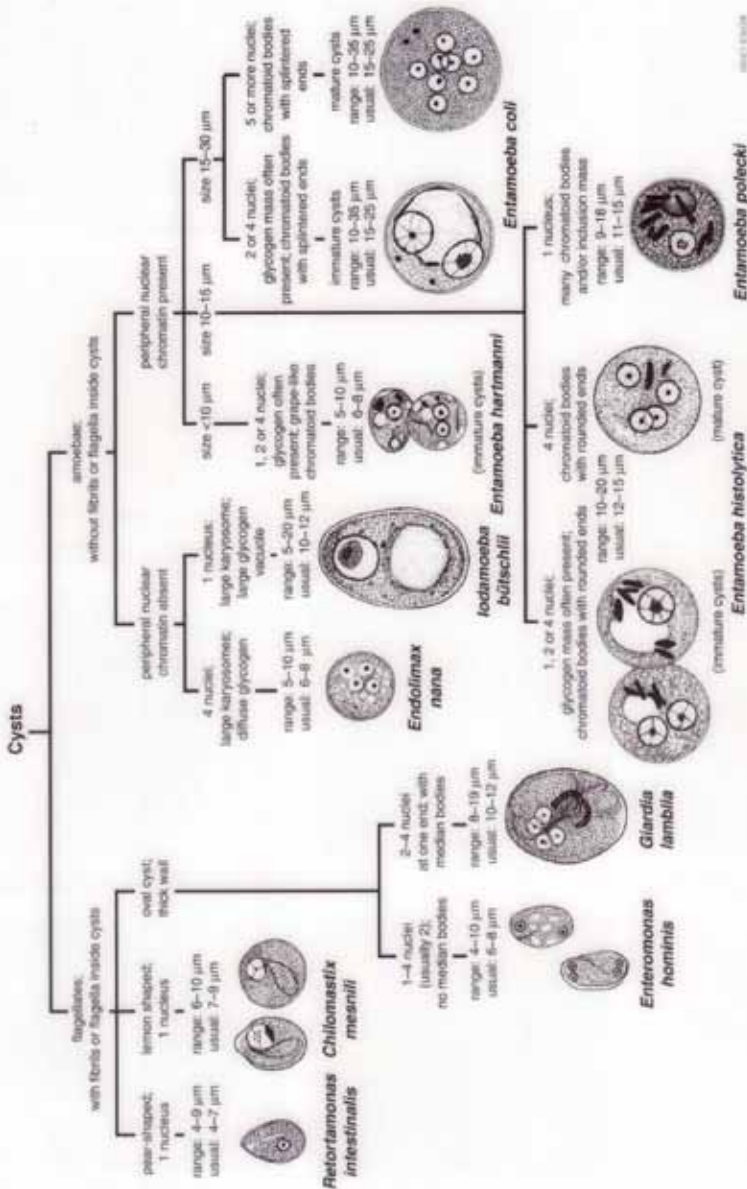
Bench Aids for the Diagnosis of Intestinal Parasites

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Plate 8

Key to identification of cysts of amoebae and flagellates in stained smears



Bench Aids for the Diagnosis of Intestinal Parasites

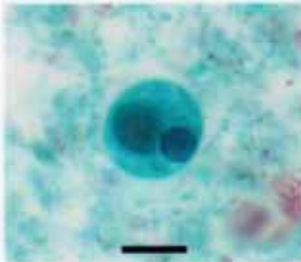
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Plate 8 – Protozoa

Note: All measuring bars = 10 µm

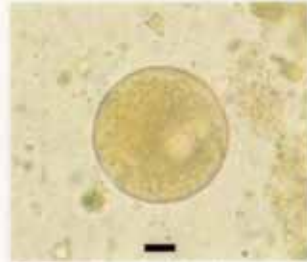
Uncommon protozoa and artefacts



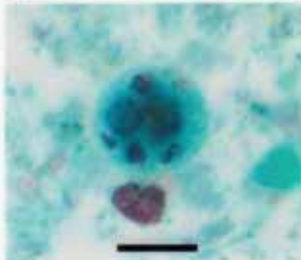
Entamoeba polecki uniloculate cyst, trichrome. Note rounded, dense inclusion mass of left side of cyst and nucleus to its right. Cysts are typically uniloculate and may or may not contain an inclusion mass.



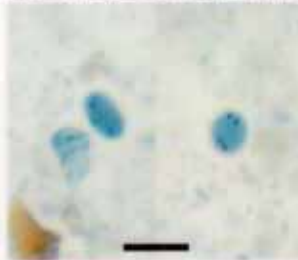
Entamoeba gingivalis trophozoite, iron haematoxylin. This amoeba has no cyst stage and is usually found in smears made from material taken from between teeth and gums. Trophozoites have *E. histolytica*-like nucleus and usually contain ingested leukocytes and bacteria.



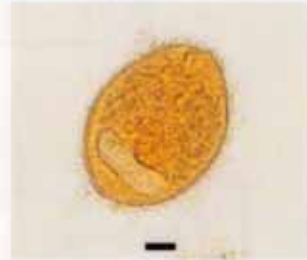
Balantidium coli cyst in unstained wet mount. Large macronucleus is visible as a clear area at right side of cyst.



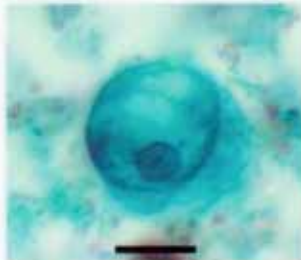
E. polecki uniloculate cyst, trichrome. Nucleus at left side is considerably obscured by large number of chromatin bodies of varying sizes. Cysts often have many chromatin bodies, with or without an inclusion mass.



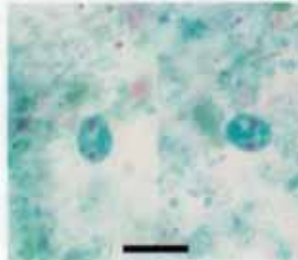
Exorhynchus hamisi trophozoite and binucleate cyst (left), iron haematoxylin. The trophozoite is to the left and the nuclei are at both ends of the cyst. The mature cyst (right) typically has 4 nuclei, 2 at each end, iron haematoxylin.



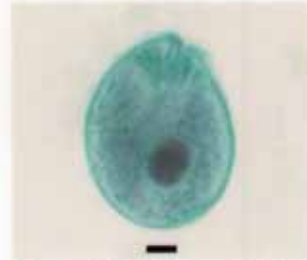
B. coli trophozoite, IIF and mount. The cytostome is visible at top of organism and the large clear area at the bottom is the macronucleus. Cilia are visible on surface.



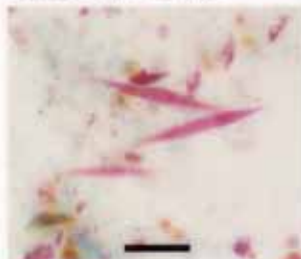
E. polecki trophozoite, trichrome. Nucleus is similar in morphology to that of *E. histolytica* trophozoite, i.e. with a small karyosome and five peripheral chromatin on nuclear membrane.



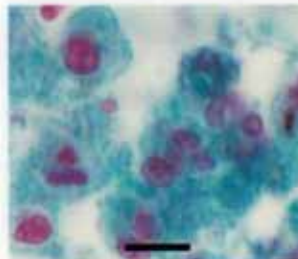
E. hamisi binucleate cysts, trichrome.



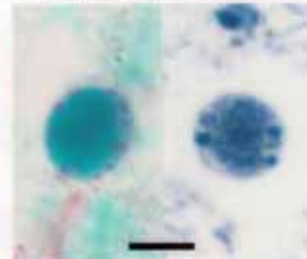
B. coli trophozoite, trichrome. The cytostome is visible at top of organism; the macronucleus is the dark-staining structure in mid-body. Cilia are visible on surface.



Charcot-Leyden crystals, trichrome. These pink-staining, elongated, pointed bodies are breakdown products of eosinophils and may often be found in faeces and sputum of patients with various types of infection.



Polymorphonuclear leukocytes are seen clustered in trichrome-stained smear. Although these may be mistaken for amoebae, the large size of nuclei in relation to the cytoplasm of the cell and their structure indicate that these are inflammatory cells.



Blastocystis hominis trichrome (left) and iron haematoxylin (right). Rounded, nucleus-like bodies, surrounding a central vacuole, are seen at periphery in both organisms.

Bench Aids for the Diagnosis of Intestinal Parasites

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Plate 9

Diagnostic features of human intestinal coccidian parasites and microsporidia

Organism	Stage in faeces	Size range	Useful stains	Other tissue sites
<i>Cryptosporidium</i> species	Sporulated oocyst	4–6 μm	Acid-fast; rhodamine-auramine O	Liver and gallbladder, respiratory epithelium; possibly other tissues
<i>Cyclospora cayentanensis</i>	Unsporulated oocyst	8–10 μm	Acid-fast	Not reported from other tissues
<i>Isospora belli</i>	Unsporulated oocyst	20–33 μm X 10–19 μm	Acid-fast	Usually not; has been reported from lung.
<i>Sarcocystis hominis</i> and <i>S. suihominis</i>	Sporulated oocysts and sporocysts	Oocysts: 15–19 μm X 15–20 μm ; Sporocysts: 15–19 μm X 8–10 μm	None	Not in humans; tissue cyst stages in other animals which are intermediate hosts
<i>Enterocytozoon bienersi</i>	Microsporidian spores	1.5 μm X 1.0 μm	Modified or "super" trichrome; calcofluor white; Warthin-Starry stain	Probably widely disseminated in body
<i>Septata intestinalis</i>	Microsporidian spores	2.2 μm X 1.2 μm	Modified or "super" trichrome; calcofluor white; Warthin-Starry stain	Probably widely disseminated in body
<i>Encephalitozoon hellem</i>	Microsporidian spores*	2.2–2.5 μm X 1.5 μm	Tissue Gram stains	Probably widely disseminated in body

* *E. hellem* spores can be found in urine but have not been reported from faeces.

Bench Aids for the Diagnosis of Intestinal Parasites

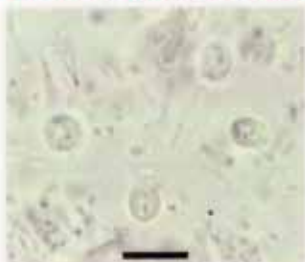
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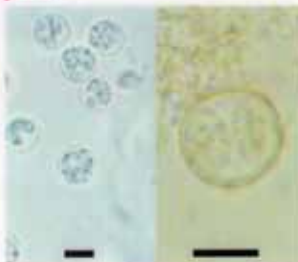
Plate 9 — Protozoa

Note: All measuring bars = 10 µm

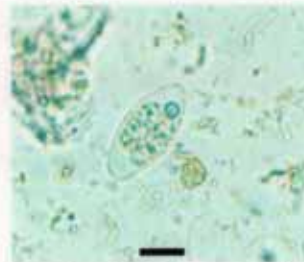
Intestinal coccidians and microspora



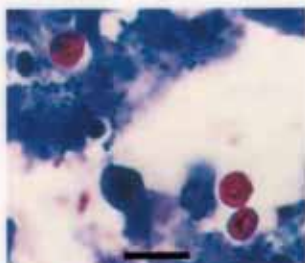
Cryptosporidium parvum oocysts, formalin wet mount. Small size (4-6 µm) and presence of black granules within oocysts are diagnostic for these organisms.



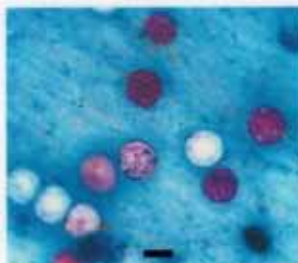
Cyclospora cayentanensis unsporulated oocysts, formalin wet mount (left). They contain numerous spherical bodies. On the right, at higher magnification, a sporulated oocyst containing 2 sporozoites.



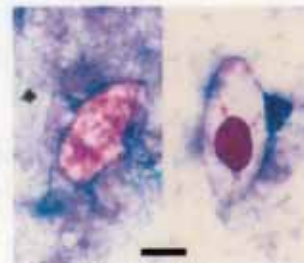
Isospora belli oocysts, formalin wet mount. Oocysts are not sporulated when excreted in faeces and are much larger than either *Cryptosporidium* or *Cyclospora*.



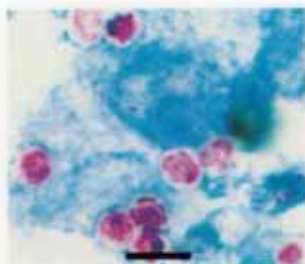
C. parvum oocysts, acid-fast. Small size, intense red coloration and presence of black granules are diagnostic for these organisms.



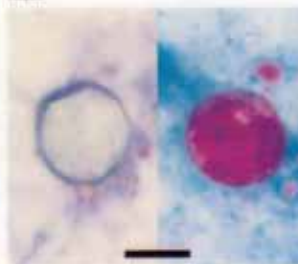
C. cayentanensis unsporulated oocysts, acid-fast. With this stain, oocysts stain variably, red, bluish, or not at all. This feature and their larger size (8-10 µm) help distinguish them from *Cryptosporidium* oocysts.



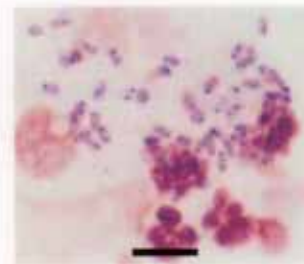
I. belli oocysts, acid-fast. On the left, atypical oocyst appears empty; these are often seen in patients undergoing treatment. On the right, typical oocyst contains red-staining sporozoites.



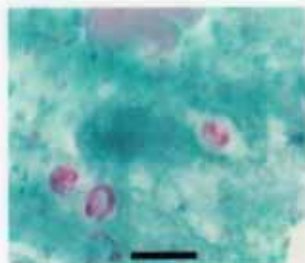
C. parvum oocysts, acid-fast. With various modifications of acid-fast stain, oocysts may stain from red to pink (as here); black granules are also seen.



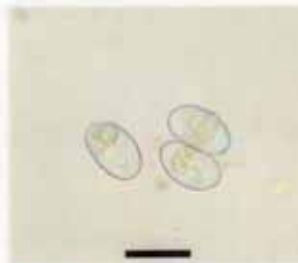
C. cayentanensis oocysts, acid-fast. The organism on the left remains unstained whereas on the right, a typical red-staining oocyst is seen.



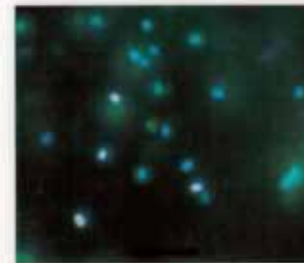
Encyrtosporidium hellem spores, Gram stain. These microsporidian spores are in urinary sediment; their small size is well demonstrated.



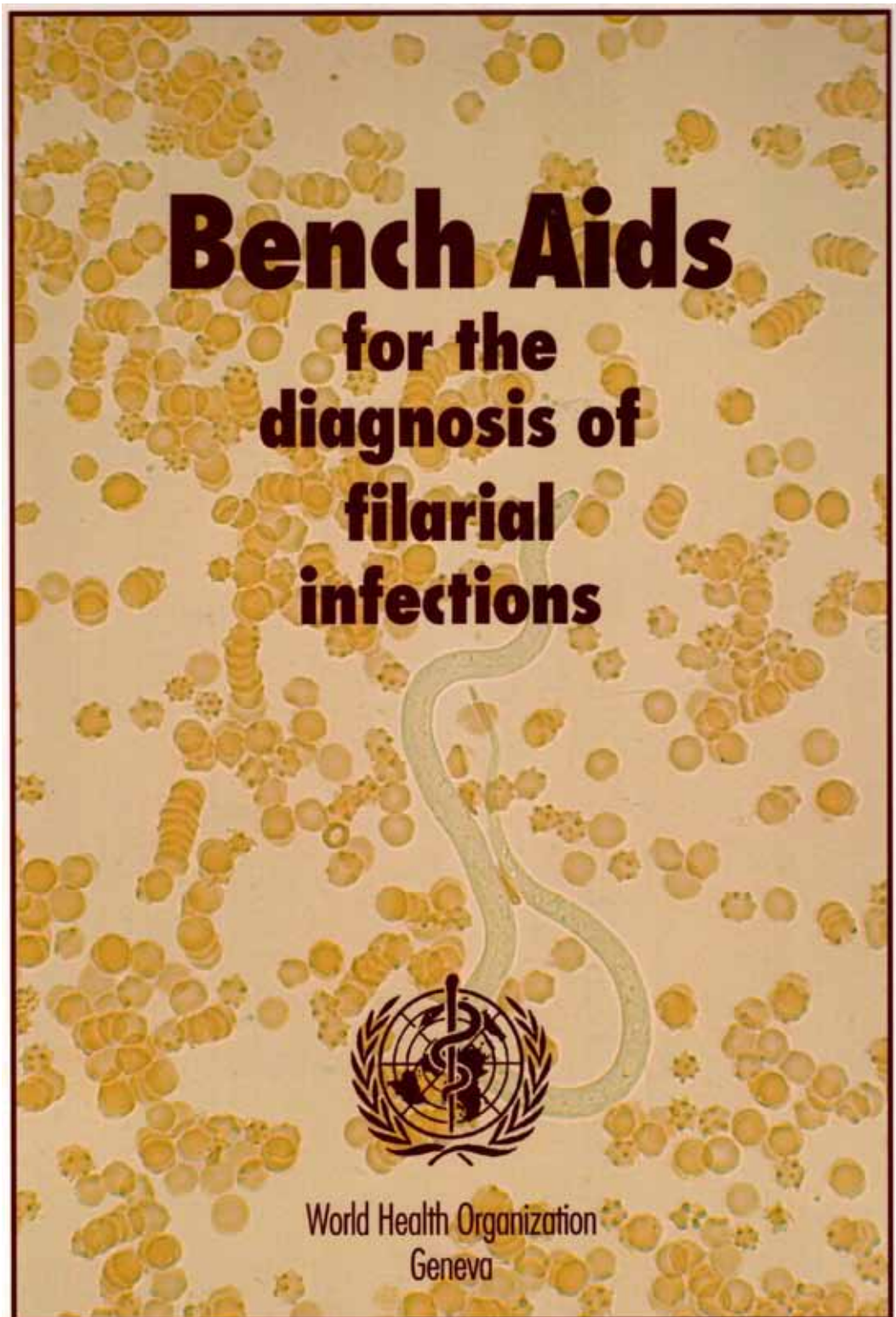
C. parvum oocysts, trichrome. Oocysts do not always stain with trichrome, but when they do, the four sporozoites within them can be seen, as illustrated here.



Sarcocystis species sporulated oocyst and sporozoist, formalin wet mount. The thin-walled oocyst contains two sporozoites but is easily ruptured so that free sporozoites are often found in faeces, as here. Sizes of all *Sarcocystis* species oocysts are very similar.



Spores of an intestinal microsporidian, ***Enterocytozoon bieneisel*** or ***Septata intestinalis***, in a calcofluor white MCH preparation with ultraviolet illumination. Small size of both species precludes specific identification in this preparation.



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Introduction

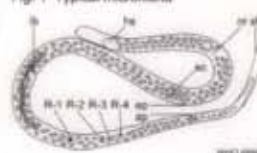
Introduction

Several species of filarial worms infect humans in the tropical and subtropical regions of the world (Table 1, overleaf). The adult worms inhabit various tissues and organs of the body and are inaccessible for identification. Consequently, diagnosis of filarial infections depends primarily on the identification of the larval stage of the parasite (microfilaria). Most species of microfilaria circulate in peripheral blood; however, some are found in the skin.

The microfilaria

At the light-microscopic level and with the aid of a variety of stains, a microfilaria appears as a primitive organism, serpentine in shape and filled with the nuclei of many cells. Figure 1 is a diagram of a typical microfilaria. In many, but not all, species, the body may be enveloped in a membrane called a sheath (**sh**). Where a sheath is present it may extend a short or long distance beyond either extremity of the microfilaria. In some species, depending on the stain used, the sheath displays a characteristic staining quality which aids in species identification. The nuclei of the cells that fill the body are usually darkly stained and may be crowded together or dispersed. The anterior extremity is typically devoid of nuclei and is called the cephalic or head space (**hs**); it may be short or long. Along the body of the microfilaria there are additional spaces and cells that serve as anatomical landmarks. These include the nerve ring (**nr**), excretory pore (**ep**), excretory cell (**ec**), and anal pore (**ap**). In some species, an amorphous mass called the innerbody (**ib**) and four small cells called the rectal cells (**R-1**, **R-2**, **R-3**, **R-4**) can be seen, usually with the aid of special stains. These structures and their positions are sometimes useful for species identification. The shape of the tail and the presence or absence and distribution of nuclei within it are also important in species identification.

Fig. 1 Typical microfilaria

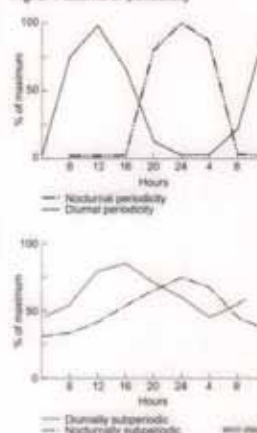


Periodicity

Some species of microfilariae circulate in peripheral blood at all hours of the day and night, while others are present only during certain periods. The fluctuation in numbers of microfilariae present in peripheral blood during a 24-hour period is referred to as periodicity (Fig. 2). Species that are found in the blood during night-time hours but are absent at other times are designated *nocturnally periodic* (e.g. *Wuchereria bancrofti*, *Brugia malayi*); those that are present only during certain daytime hours are designated *diurnally periodic* (e.g. *Loa loa*). Microfilariae that are normally present in the blood at all hours but whose density increases significantly during either the night or the day are referred to as *subperiodic*. Microfilariae that circulate in the blood throughout a 24-hour period without significant changes in their numbers are referred to as *nonperiodic* or *aperiodic* (e.g. *Mansonella* spp.).

The periodicity of a given species or geographical variant is especially useful in determining the best time of day to collect blood samples for examination. To determine microfilarial periodicity in an individual, it is necessary to examine measured quantities of peripheral blood collected at consecutive intervals of 2 or 4 hours over a period of 24–30 hours.

Fig. 2 Patterns of periodicity



Further reading

Basic laboratory methods in medical parasitology. Geneva, World Health Organization, 1991.

Ash LR, Orihel TC. Atlas of human parasitology, 4th ed. Chicago, ASCP Press (in press).

Ash LR, Orihel TC. Parasites: a guide to laboratory procedures and identification. Chicago, ASCP Press, 1991.

Orihel TC, Ash LR. Parasites in human tissues. Chicago, ASCP Press, 1995.

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Table 1. Characteristics of common human filarial parasites

Species	<i>Wuchereria bancrofti</i>	<i>Brugia malayi</i>	<i>Brugia timori</i>	<i>Loa loa</i>	<i>Mansonella ozzardi</i>	<i>Mansonella perstans</i>	<i>Mansonella streptocerca</i>	<i>Onchocerca volvulus</i>
Geographical distribution	Tropics and subtropics worldwide	South-east Asia, Indian subcontinent	Indonesian archipelago, Timor, Lesser Sunda Islands	West and Central Africa	Caribbean, Central and South America	Africa and South America	West and Central Africa	Africa, Yemen, Central and South America
Vectors	Mosquitoes: Culex, Aedes, Anopheles, Mansonia	Mosquitoes: Mansonia, Anopheles, Aedes	Mosquitoes: Anopheles	Tsetse flies: Chrysops	Biting midges: Culicoides, Black flies, Simulium	Biting midges: Culicoides	Biting midges: Culicoides	Black flies: Simulium
Adult habitat	Lymphatic system	Lymphatic system	Lymphatic system	Subcutaneous tissues, conjunctivae	Subcutaneous tissues	Mesenteries, connective tissues of abdominal organs	Dermis	Subcutaneous and deeper tissues
Habitat of microfilaria	Blood	Blood	Blood	Blood	Blood	Blood	Skin	Skin
Periodicity	Nocturnal ^a	Nocturnal	Nocturnal	Diurnal	Aperiodic	Aperiodic	—	—
Sheath	Present	Present	Present	Present	Absent	Absent	Absent	Absent
Length (µm) ^b	244–266 (260) 275–317 (298)	177–230 (220) 240–298 (270)	265–323 (287) 332–383 (358)	231–250 (238) 270–300 (281)	163–203 (183) 203–254 (224)	190–200 (195) 183–225 (203)	— 180–240 (210)	— 304–315 (309)
Width (µm)	7.5–10.0	5.0–6.0	4.4–6.8	5.0–7.0	3.0–5.0	4.0–5.0	5.0–6.0	5.0–9.0
Tail	Tapered; anucleate	Tapered; subterminal and terminal nuclei widely separated	Tapered; subterminal and terminal nuclei widely separated	Tapered; nuclei irregularly spaced to end of tail	Long, slender; pointed; anucleate	Bluntly rounded; nuclei to end of tail	Bluntly rounded; bent into hook; nuclei to end of tail	Typically filarid, tapered to a point; anucleate
Key features of microfilaria	Short head space; dispersed nuclei; sheath unstained in Giemsa; body in smooth curves	Long head space; sheath stains pink in Giemsa; terminal and subterminal nuclei	Long head space; sheath unstained in Giemsa; terminal and subterminal nuclei	Single row of nuclei to end of tail; sheath unstained in Giemsa	Small size; long slender tail; aperiodic	Small size; blunt tail filled with nuclei; aperiodic	Slender shape; hooked tail filled with nuclei; occurs in skin	Fleuret tail; occurs in skin, occasionally in urine or blood after treatment

^a Reported in Brazil, Guyana, and the Amazon region of Colombia.
^b Diurnally subperiodic in New Caledonian and Polynesian regions; exclusively subperiodic in rural areas of Thailand.
^c Nocturnally subperiodic in parts of Indonesia, Malaysia, Philippines, and Thailand.
^d Mean values given in parentheses.

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Plate 1

Diagnosis of filarial infections

As well as in blood and skin, microfilariae may occasionally be found in bone marrow preparations, fine-needle biopsy aspirates, cervical smears contaminated with blood, hydrocele fluid, chylous urine, and normal urine following treatment with diethylcarbamazine. Methods commonly used for the detection of microfilariae include:

Blood examination

- stained thick blood films
- direct examination of capillary blood
- membrane filtration (fresh or preserved blood)
- haemolysed venous blood concentration (Knott concentration method)

Tissue examination

- skin snips

Other body fluid examination

- urine
- hydrocele fluid

Caution: Standard biosafety guidelines should be followed in obtaining blood and tissue samples. Disposable or sterile lancets, syringes, and needles should be used for all laboratory procedures. These guidelines are summarized in *Biosafety guidelines for diagnostic and research laboratories working with HIV* (Geneva, World Health Organization, 1991; WHO AIDS Series, No. 9).

Preparation of thick blood films

The examination of thick blood films is the most widely used method in field surveys of filarial infection. Properly done, it is a reliable procedure for both identification of microfilariae and enumeration studies. Carefully measured samples of at least 20 µl and preferably 60 µl in volume are recommended.

1. Thoroughly clean the microscope slides (including factory "pre-cleaned" slides) before use. Dust, grease, detergent, or cotton lint and threads may cause the blood film to lift off the slide.
2. Clean the finger tip (or ear lobe) from which the blood will be taken with a cotton ball soaked in alcohol.
3. Prick the finger tip or ear lobe with a sterile lancet and allow the blood to ooze freely.
4. Draw the required volume of blood into a disposable or sterile calibrated capillary pipette.
5. Expel the blood onto a microscope slide and smear the sample uniformly in a circular or rectangular shape; avoid creating any bubbles.
6. Allow the slide to dry at room temperature in a horizontal position.
7. Label and store the slide in a dust-free environment until staining. It is also important to protect unfixed blood films from damage by insects.

Note: Excess alcohol on the skin may partially fix the blood sample; squeezing the finger or ear lobe may dilute the sample with tissue fluids. Films that are too thick tend to lift off the slide. Blood films must be thoroughly dried before dehaemoglobinization; this may require 12–48 hours, depending on humidity. If blood is collected in a heparinized capillary pipette, or if the film is made from blood containing an anticoagulant, drying requires at least 48–72 hours. Thin blood films are of little value because the volume of blood examined is small. However, when microfilariae are found in thin films they tend to be concentrated at the "feathered" end and at the margins of the film. The morphology of microfilariae found in thin films tends to be good since the films are routinely fixed before staining.

Capillary blood examination

Microscopic examination of fresh blood has limited utility. It can reveal the presence of microfilariae actively moving among the red blood cells (see front cover), but species identification is not possible. However, in regions where only one species of microfilaria is found, its presence and density in the blood can be determined with reasonable accuracy by this means.

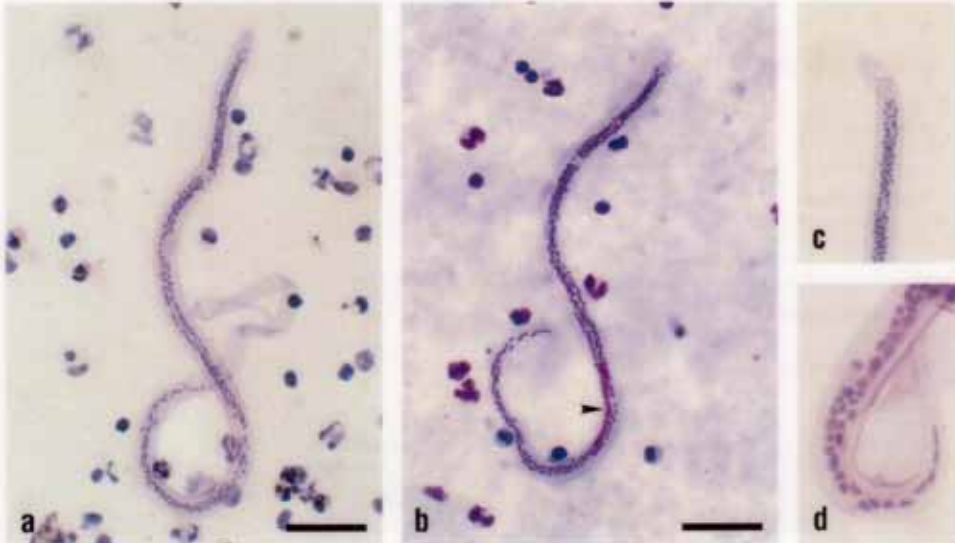
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Plate 1 – *Wuchereria bancrofti*, *Loa loa*

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Note: All measuring bars = 30 µm



Wuchereria bancrofti microfilariae in haematoxylin (a, c, d) and Giemsa (b) stains. Characteristically, the sheath stains lightly with haematoxylin (a, c) but not with Giemsa stain (b). Key morphological features include a short head space (a, b, c) and discrete nuclei in the body. The column of nuclei does not extend to the end of the tail (d). The innerbody stains pink with Giemsa stain (b, arrowhead) but not with haematoxylin stain.



Loa loa microfilariae in haematoxylin (e) and Giemsa (f–h) stains. The sheath is clearly evident in haematoxylin (e) but not in Giemsa stain; however, in Giemsa stain, its presence is often demarcated by red blood cells that lie along the margin of the sheath (f). Key features of ***L. loa*** include a short head space (g) and a compact column of nuclei that extends to the end of the tail; the last few nuclei are irregularly spaced (h). Very frequently, the tail is flexed or coiled within the sheath (e, inset). There is no easily identifiable innerbody in stained ***L. loa*** microfilariae.

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Plate 2

Staining of thick blood films

Giemsa and haematoxylin are the preferred and most widely used stains for preparing permanently stained blood films. Each has its advantages, but Giemsa stain is used most often. Slides can be processed in either small or large numbers using stainless steel, glass, or plastic staining racks and dishes.

Before staining, thoroughly dried films must be dehaemoglobinized and fixed. Immerse slides in tap or distilled water until the haemoglobin leaches out of the film, which becomes whitish in colour; this requires about 3–5 minutes. Films that are prepared from blood containing an anticoagulant and that have dried for more than a few days will dehaemoglobinize slowly, usually in 6–10 minutes. Allow dehaemoglobinized films to air-dry thoroughly. Fix the films in methanol for 30–60 seconds and air-dry.

Note: In the event that the same films are being used for malaria surveys, they should be stained without dehaemoglobinization or fixation. Microfilariae found in these preparations usually appear slightly swollen, and the nuclei are not sharply demarcated (Plate 3b).

Giemsa stain

Stain blood films for 45 minutes in a 1:50 dilution of Giemsa stain (or 20 minutes in a 1:20 dilution) at a pH of 6.8–7.2; wash films for 3–5 minutes in neutral buffered water or under running tap water. Dry films in a vertical position.

Note: The staining dilution and procedure used for processing malaria films can be used here with the expectation of acceptable results. Nuclei of microfilariae will stain blue to purple in colour. A sheath, if present, will stain pink (*B. malayi*) or not at all. The innerbody of *W. bancrofti* will stain a bright pink colour, but that of most other species does not stain.

Haematoxylin stain

Various haematoxylin stains are used as alternatives to Giemsa stain; Delafield's haematoxylin is recommended and is widely used. It enhances nuclear detail in the microfilaria and stains the sheath, when present, a greyish-blue colour. For preparation of Delafield's haematoxylin and details of another useful staining procedure, consult the WHO publication *Basic laboratory methods in medical parasitology* (1991). It is also acceptable to use other available stains and procedures.

Procedure

1. Thick blood films should be dried thoroughly, dehaemoglobinized, and fixed as described above. If films are prepared from sedimented Knott concentration material, dehaemoglobinization and fixation are omitted.
2. Stain slides for 10–15 minutes in Delafield's haematoxylin solution. Rinse in distilled water to remove excess stain.
3. Destain in 0.1% (1 g/l) aqueous hydrochloric or acetic acid for approximately 1 minute. Rinse slides in distilled water for 1 minute.
4. Place the slides in tap water containing several drops of ammonia for 3–5 minutes. The films will become dark blue in colour.
5. Rinse in tap water for 2–5 minutes and allow to dry.

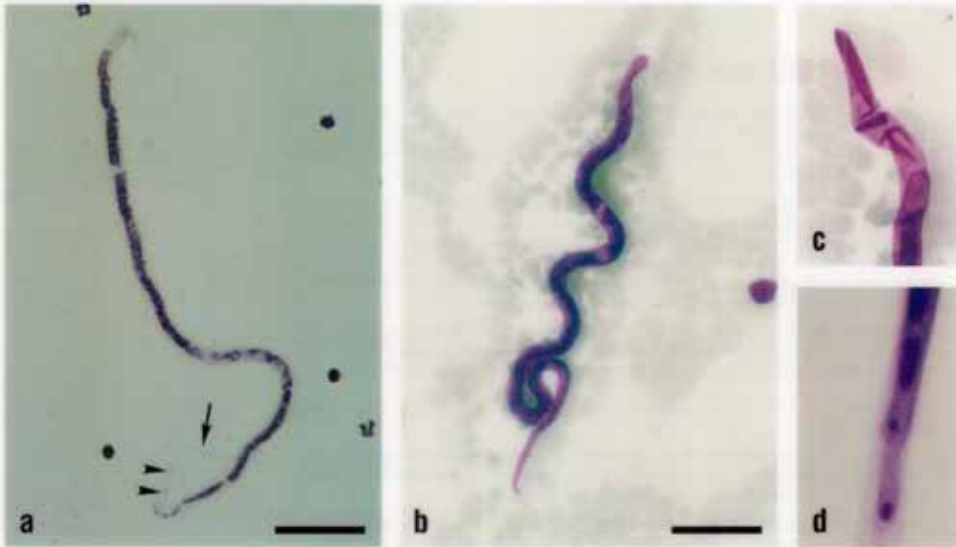
Note: Films may be made permanent by adding a synthetic mounting medium and a coverslip. Alternatively, simply clarify with a drop of immersion oil, add a coverslip, and examine under low magnification.

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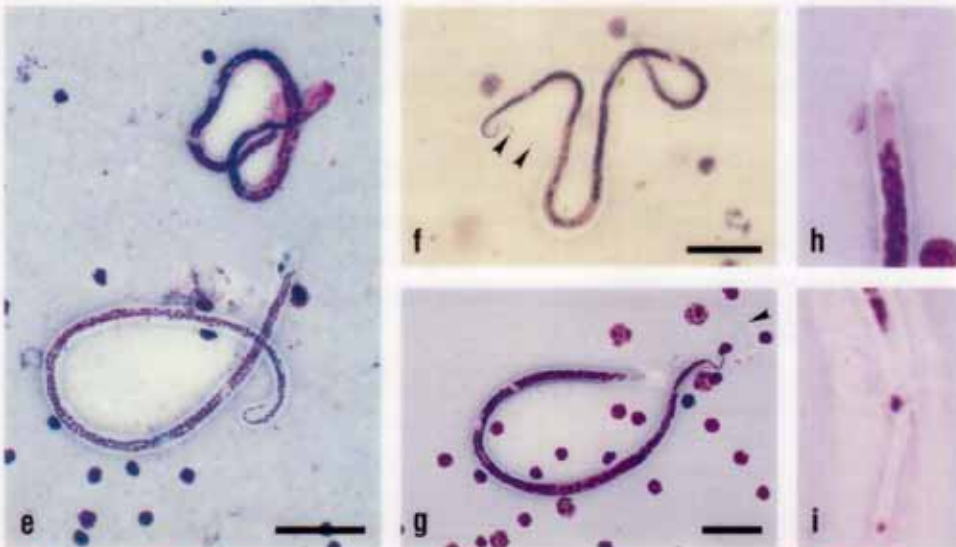
Plate 2 — *Brugia malayi*, *Brugia timori*

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Note: All measuring bars = 30 µm



Brugia malayi microfilariae in haematoxylin (a) and Giemsa (b–d) stains. In haematoxylin, the sheath does not stain but may be faintly visible (a, arrow). This contrasts with the pink-stained sheath seen in Giemsa preparations (b, c). The column of nuclei is compact, and the widely separated subterminal and terminal nuclei in the tail are key diagnostic features (a, arrowheads; d). Nuclei are sparse in the region of the innerbody (a).



B. malayi (upper) and ***W. bancrofti*** (lower) microfilariae in the same field of a Giemsa-stained blood film (e). The pink-stained sheath and the darkly stained, compact column of nuclei identify ***B. malayi*** and distinguish it from ***W. bancrofti***.

Brugia timori microfilariae in haematoxylin (f) and Giemsa (g–i) stains. ***B. timori*** is larger than ***B. malayi*** and the sheath does not stain pink (g, arrowhead) with Giemsa stain. The long head space and the subterminal and terminal nuclei are conspicuous features (f–i).

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Plate 3

Concentration procedures

The detection of microfilariae in peripheral blood when few are present is best accomplished by concentration procedures, which allow for the examination of a larger volume of blood. The use of membrane filtration and the Knott concentration method are the most widely used procedures.

Membrane filtration

Membrane filtration allows for removal of elements in the blood by filtration through a membrane of desired pore size. Membrane filtration is more effectively used to determine microfilarial density than as a means of microfilaria identification. Cellulose-mixed-ester filters (e.g. Millipore filters) and polycarbonate filters (e.g. Nuclepore filters) are the most common membrane filters used. Formerly, fresh blood samples required processing soon after they were obtained. Recently, however, a procedure for membrane filtration of preserved blood has been published (1). Both are described below.

Filtration of fresh whole blood

Materials and reagents

1. Sodium citrate solution, 3.8% (36 g/l) or EDTA (ethylenediaminetetraacetic acid) solution, 7.5% (75 g/l).
2. Teepol-saline solution, 10% (prepare by adding 50 g Teepol concentrate to 450 ml saline).
3. Saline, 0.85% (8.5 g/l).
4. Giemsa stain.
5. Syringe (disposable polypropylene with rubber plunger tip), 20-ml capacity.
6. Membrane filter holder (e.g. Swinnex type).
7. Membrane filter, 3–5- μ m porosity, 25-mm diameter.

Note: Although a pore size of 5 μ m is ideal for *L. loa* microfilariae, 4 μ m is more efficient for filtration of *W. bancrofti* and other smaller species of microfilariae such as *Mansonella perstans*.

8. Absolute methanol.

Procedure

1. Collect a fresh blood sample in sodium citrate or EDTA solution.
2. Add 1 ml of citrated or EDTA-preserved blood to 10 ml of Teepol-saline solution.
3. Place moistened membrane filter, secured with a rubber gasket, into filter holder (Fig. 3).
4. Remove plunger from barrel of 20-ml syringe and connect barrel of syringe to filter holder.
5. Pour the blood-Teepol mixture (from step 2) into barrel of syringe, replace plunger in syringe and, by applying gentle, even pressure, force solution through filter (Fig. 4). Discard blood into disinfectant for disposal.

Note: Some workers prefer to push a 1-ml blood sample directly through the filter followed by 20–35 ml of water or saline to wash out the remaining blood. Others suspend the blood in 10 ml of water, agitate, and allow the mixture to stand for several minutes before passage through the filter.

6. Remove syringe from filter holder, draw up 10 ml of water into syringe, reattach filter holder, and gently wash filter by flushing the solution through it.
7. Force two syringe-volumes of air through filter to expel excess water and make microfilariae more adherent to filter.

Note: Procedures may be modified at this point depending on the type of preparation desired.

Microfilariae may be fixed and stained on the filter as follows:

8. For permanent, stained preparations:
 - a. Pass 3 ml of methanol through filter to fix microfilariae.
 - b. Pass air through filter to expel residual methanol.
 - c. Remove filter from the holder and place on a glass slide; allow it to dry thoroughly.
 - d. Stain the preparation in Giemsa stain as for a blood film.
 - e. Rinse in tap water and allow to dry.
 - f. Dip the slide in toluene to avoid bubbles in or under the filter. Add a drop of synthetic mounting medium and a coverslip. The slide may be examined in the same manner as any blood film and stored as a permanent preparation for future reference.

Microfilariae may be examined alive as follows:

9. Alternatively, following step 7, remove syringe from filter holder, carefully unscrew top from filter, and, using forceps, remove rubber gasket.
10. Use fine forceps to transfer wet filter to a slide, with the residue on the membrane facing upwards.
11. Add a drop of saline to the membrane and cover with a coverslip. Examine under the microscope with 10x objective; microfilariae will be seen actively moving.

Fig. 3

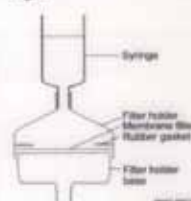


Fig. 4



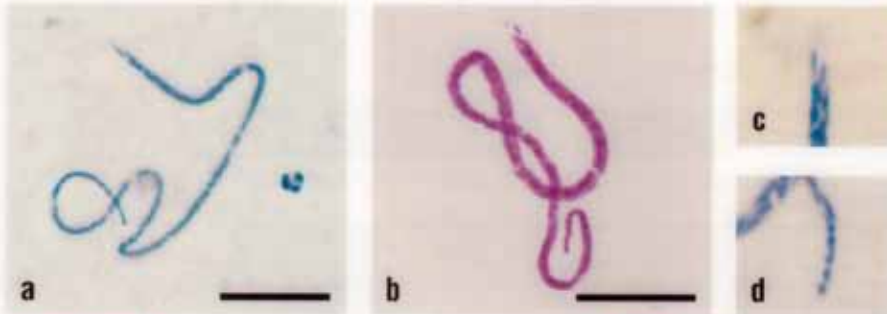
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Plate 3 – *Mansonella perstans*, *Mansonella ozzardi*

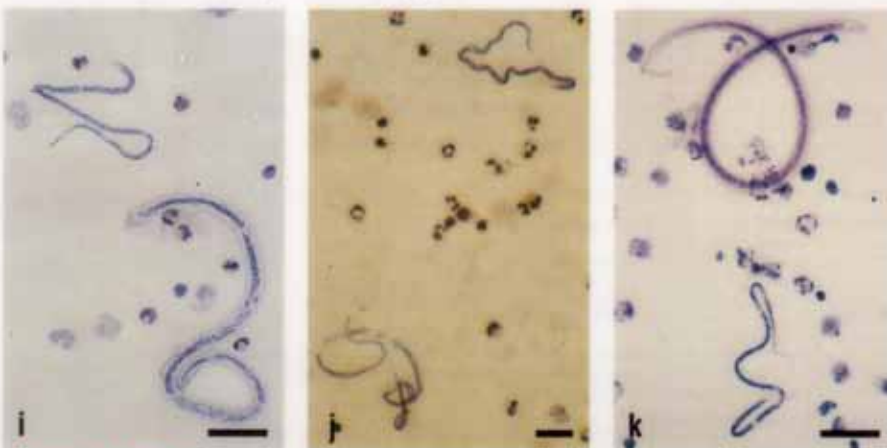
Note: All measuring bars = 20 µm



Mansonella perstans microfilariae in haematoxylin (a, c, d) and Giemsa-stained (b) blood films. *M. perstans* is small, has a short head space (c), lacks a sheath, and is readily recognized by the blunt tail that is filled by the column of nuclei (a, b, d). In thick blood films stained with Giemsa stain without fixation, the body usually appears thickened, and individual nuclei may be indistinct (b).



Mansonella ozzardi microfilariae in haematoxylin (e, g, h) and Giemsa (f) stains. Key features of this small, unsexed microfilaria include a compact column of nuclei, a head space that is slightly longer than it is wide (g) and, most importantly, a tail that is long, slender, and devoid of nuclei (h). The appearance is the same in haematoxylin and Giemsa stains (e, f).



M. perstans and *M. ozzardi* are often found in individuals infected with other filariae in areas where species overlap. It is not uncommon to see, as in (i), *M. perstans* (upper) with *L. loa* (lower), more rarely, as in (j), *M. perstans* with *Microfilaria zimmermanni* (lower).¹ In the Americas, as shown in (k), mixed infections of *M. bancrofti* (upper) and *M. ozzardi* are often seen. Mixes of *M. perstans* and *M. ozzardi* are also common. Microfilariae (i–k) stained in haematoxylin.

¹*Microfilaria zimmermanni* (j) has been found in the blood of people in Zaire (Faint A. *Diplosomella zimmermanni* sp. nov. from the blood of man in the Republic of Zaire (Congo-Kinshasa). *Parasitica*. *Annales de la Société belge de Médecine tropicale* 1974, 54:195–207). A valid genus name has not been assigned to this species of filarial worm.

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Plate 4

Concentration procedures (continued)**Filtration of preserved blood**

If it is not possible to process fresh blood immediately, the following procedure may be used.

Materials and reagents

Materials required are the same as for processing whole blood, except that 2 ml of 37% (370 g/l) formaldehyde solution is added to 10 ml of Teepol concentrate and 88 ml of distilled water to make 100 ml of Teepol-formalin solution.

Procedure

1. Blood specimens preserved in the Teepol-formalin solution (1 ml of blood should be added to 10 ml of Teepol-formalin solution) are filtered through a membrane filter in the same manner as described in steps 3–7, above.
2. Filters can be examined wet, with or without the addition of Giemsa, haematoxylin, or other stains, to allow for enumeration of the microfilariae or study of their morphology.
3. Alternatively, the wet filter can be placed on a slide, allowed to dry, and stained as desired. A drop of synthetic mounting medium and a coverslip can be added to make a permanent preparation.

Note: Teepol lyses blood and formaldehyde preserves the morphological features of microfilariae. Blood specimens in the Teepol-formalin solution can be retained for 9 months or longer before examination, without marked deterioration of the microfilariae. Blood specimens in Teepol only, or in a Teepol-sodium azide solution, are not useful for long-term storage since microfilariae undergo degenerative changes within a week or less.

Knott concentration method

The Knott concentration method is very sensitive and relatively inexpensive to perform.

Materials and reagents

1. Centrifuge tubes, 15-ml capacity.
2. Formalin, 2% (2 ml of 37% (370 g/l) formaldehyde solution + 98 ml of distilled water).
3. Slides and coverslips.
4. Needles and syringes.
5. Centrifuge (hand- or electric-powered).

Procedure

1. Collect 1 ml of blood (whole or citrated) by venepuncture and place in a 15-ml centrifuge tube containing at least 10 ml of formalin; shake vigorously. Red cells are lysed by the formalin solution.
2. Centrifuge at approximately 300g for 2 minutes. If a centrifuge is not available, place the tube in an upright position for 12 hours for gravitational sedimentation.
3. Decant the supernatant fluid (the small amount remaining in the tube is allowed to flow back on to the sediment).
4. Examine a drop of the sediment on a slide under a coverslip with the low-power objective of the microscope.
5. A portion of the sediment may be spread on a slide as a thick film and allowed to dry thoroughly. Stain the film with Giemsa or haematoxylin stain.

Note: Avoid adding more than 1 ml of blood to 10 ml of formalin, as much as 12–14 ml of formalin is desirable for each 1 ml of blood. Only microfilariae and white blood cells are found in the sediment; microfilariae are fixed without significant shrinkage and are easy to count accurately. A sheath, if present, is also easy to see. The technique is useful for quantification of microfilaraemia. Samples need not be examined immediately and can be stored in the laboratory for several weeks. Microfilariae present in the stained sediment will show details of internal structure.

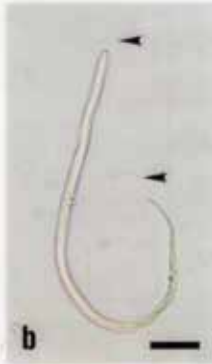
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Plate 4 – Techniques, artefacts, oddities

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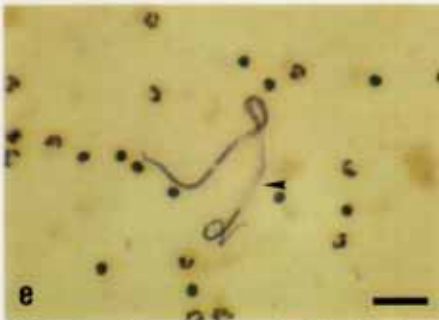
Note: All measuring bars = 30 µm



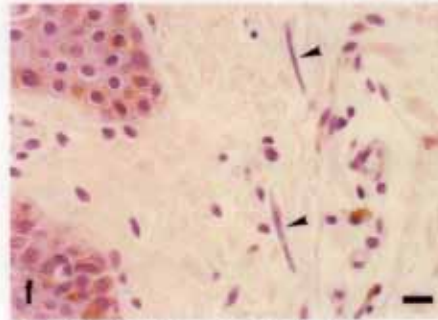
L. loa microfilariae in a Knott concentration. They are easily enumerated at low magnification (a). At high magnification, features such as size, shape, and the presence or absence of a sheath are evident (b). Note the sheath extensions (arrowheads) at both ends (b).



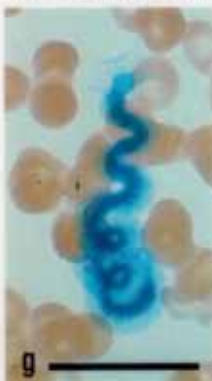
L. loa microfilariae collected on a polycarbonate filter and stained with Giemsa stain. Microfilariae are easily enumerated at low magnification (c); the distribution of nuclei in the tail allows the identification of the microfilaria at high magnification (d).



Microfilaria ***somaticum*** superficially resembles ***M. perstans***; it is similar in size but has a sparsely nucleated area (arrowhead) in the posterior half of the body. The adult worms and vectors have not been identified. Preparation stained in haematoxylin.



O. volvulus microfilariae in a section of skin stained with haematoxylin and eosin. Only portions of the microfilariae are visible (arrowheads).



Fibres (g, h), unidentified elements (i), and fungi (***Helicospirium***) (j) found on Giemsa-stained blood films are often confused with microfilariae. In spite of similar size, the presence of a darkly stained core and/or vacuoles, the absence of nuclei, and jagged or broken ends rule out identification as microfilariae.

Bench Aids for the diagnosis of filarial infections

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Plate 5

Tissue examination

Skin snips

The microfilariae of *Onchocerca volvulus* and *Mansonella streptocerca* that reside in the skin are best detected by looking for their presence in skin snips. Intensity of infection is reflected in the numbers of microfilariae emerging from the snips. Skin snips are obtained in one of two ways:

1. Skin snips can be standardized in both size and weight through the use of sclerocorneal punches of either the Holth or Walser type. These instruments take snips of uniform diameter (approximately 2.3–2.5 mm). This is the preferred method.
2. A needle can be used to raise the skin and a razor blade to cut off the raised area; forceps and curved scissors can also be used. Such skin snips vary in size, shape, and the depth of the cut. When snips are cut too deeply, small capillaries may be lacerated and the snip may be contaminated by microfilariae that might be present in the patient's blood.

Caution: It is of the utmost importance that all instruments used for each patient are sterile in order to avoid transmission of viral hepatitis B and HIV infections.

Procedure

1. Skin snips should be taken from selected sites on the body. In Africa, the preferred site is the iliac crest; in Central and South America, the iliac crest or the scapular area; and in Yemen, the lower calf. In surveys, ideally two snips should be taken from all three of these sites on each side of the body of the individual.
2. Transfer skin snips from each site to a drop of normal saline, distilled water, or tissue culture medium in a well of a 96-well, flat-bottom, tissue culture tray; or place snips on a microscope slide in one of the fluids. It is not necessary to tease the snips.
3. Examine after 30 minutes to 3 hours. (Tissue culture trays may be covered with plastic wrap or similar material, and slides placed in a covered Petri dish, to retard evaporation.) If the wells or slides are negative for microfilariae, allow the snips to remain overnight in an incubator at 37 °C or at room temperature and examine them again. If microfilariae are present they will be apparent in the fluid. The morphological features of *O. volvulus* and *M. streptocerca* are so distinct that differentiation of microfilariae is quite easy.
4. To make permanent preparations of microfilariae, remove the skin snips, transfer the fluid to a slide if necessary, and allow the fluid to evaporate. When the slide is thoroughly dry, fix the microfilariae in methanol and stain with Giemsa or haematoxylin stain.

Urine and hydrocele fluid

Pour 15 ml of urine or hydrocele fluid into a conical centrifuge tube and centrifuge for 5 minutes at 350g or more. Pour off supernatant and examine sediment for microfilariae. Slides can be stained and/or fixed as described for blood samples.

Other diagnostic methods

Microhaematocrit

Originally used for diagnosis of trypanosomiasis, the microhaematocrit procedure is equally useful for the diagnosis of filarial infections, especially when the numbers of microfilariae present are too small for efficient detection by thick blood films. Only a small amount of blood is needed, so that one or two drops obtained by finger-prick can be used when venepuncture cannot be performed (2).

Quantitative buffy coat

The utilization of the quantitative buffy coat tube (microhaematocrit tube recoated with acridine orange) has been reported to be an acceptable rapid diagnostic test for the detection of microfilariae, with a sensitivity equivalent to that of the thick blood film (3).

Microfilariae counts

Accurate counts of microfilariae can be made from stained thick blood films of measured volume. Counting requires careful systematic scanning of the blood film with the low-power objective of the microscope. The stained slides can be kept as a permanent record. Equally reliable counts can be made from membrane filters which, if mounted with a coverslip, can be retained as a permanent record. Some investigators prefer using a counting-chamber technique, which is very reliable but does not lend itself to species identification or permanence (4).

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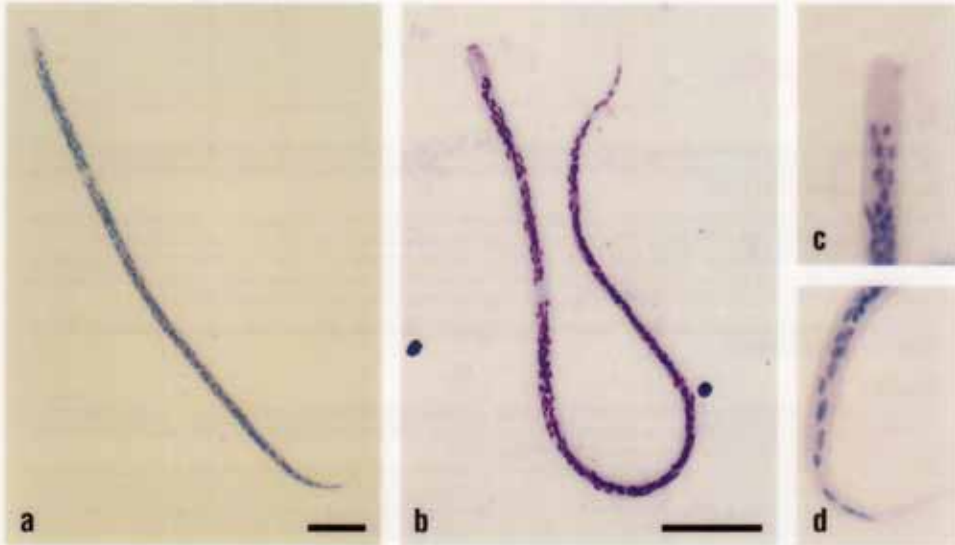
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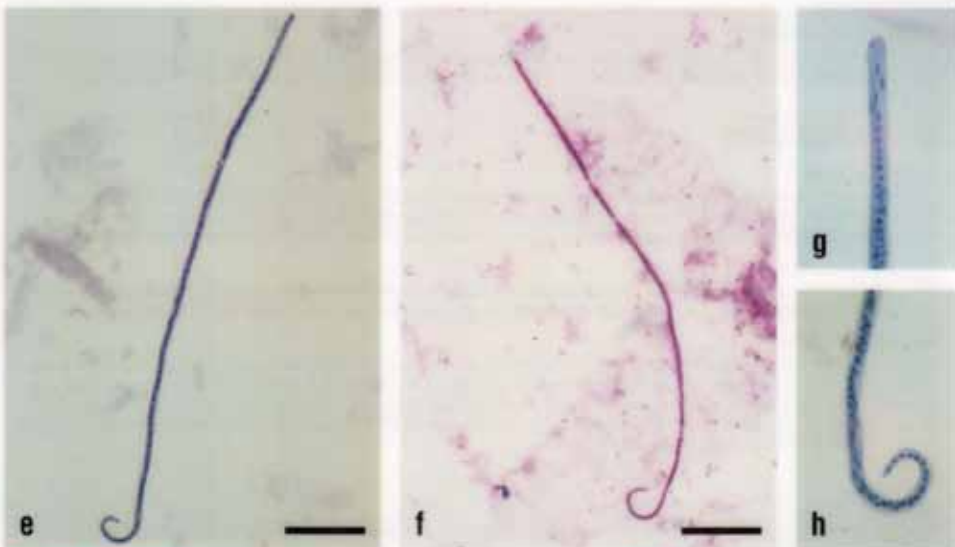


Plate 5 – *Onchocerca volvulus*, *Mansonella streptocerca*

Note: All measuring bars = 20 µm



Onchocerca volvulus microfilariae from skin snips in haematoxylin (a, c, d) and Giemsa stains (b). This microfilaria is large and has no sheath, a long head space (c) and, typically, a flexed tail (d). The column of body nuclei is only moderately compact. The most important diagnostic feature is that ***O. volvulus*** is found in the skin and only rarely in the blood.



Mansonella streptocerca microfilariae from skin snips in haematoxylin (e, g, h) and Giemsa stains (f). ***M. streptocerca*** is readily distinguished from ***O. volvulus*** by its very slender shape and "crooked" tail (e, f, h). Note that the column of nuclei starts in the anterior extremity as a single row of 10–12 (or more) nuclei (g) and extends to the end of the tail (e, f, h).

Bench Aids for the diagnosis of malaria infections

Second edition



World Health Organization
Geneva

Bench Aids for the diagnosis of malaria infections

Plate 1

Introduction

About 300–500 million people worldwide are infected with malaria each year, and more than 1 million, mainly children, die from the disease. Most cases occur in developing countries, particularly in Africa. Malaria is caused by protozoan parasites of the genus *Plasmodium*, which contains four species important to humans: *P. falciparum* (which causes the most serious form of malaria and is responsible for the majority of deaths), *P. vivax*, *P. ovale* and *P. malariae*. The parasite attacks and destroys red blood cells, and it may affect vital body organs, including the brain: most deaths due to *falciparum* malaria are the result of cerebral malaria.

These Bench aids for the diagnosis of malaria infections present photomicrographs — with explanatory text — that show the various species and morphological forms of human malaria in thick and thin blood films. Descriptions of *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* are provided, as are instructions on the preparation and use of buffer and staining solutions. The photomicrographs, all at x1000 magnification and stained with Romanowsky stains, show many of the possible variations of malaria parasites. Differences in fixing and staining procedures result in highly variable staining of blood films; as a result, the background of the film and the organisms will differ considerably from slide to slide. Parasites in the photomicrographs of thick blood films tend to be less clearly defined than those in thin films: a thick film consists of several layers of cells, making it difficult to focus on different layers.

These bench aids are aimed at laboratory workers responsible for diagnosing malaria by microscopic examination of blood films, but will also be useful as teaching aids. Experienced health workers can often diagnose malaria from the way a patient looks and feels. To confirm the diagnosis, however, the patient's blood must be examined: for microscopy thick and thin blood films should therefore be prepared. A thick blood film usually allows an experienced microscopist to identify the species of *Plasmodium*, but where there is any doubt a thin blood film should be examined to confirm the identity. Accurate diagnosis is essential, since treatment varies according to species; diagnostic errors can be life-threatening for patients, particularly in *P. falciparum* infection.

The previous edition of the bench aids (*Bench aids for the diagnosis of malaria*), published by WHO in 1988, included colour illustrations of the different life-cycle stages of the four *Plasmodium* species that cause human malaria. In this edition, colour micrographs, which provide a more accurate representation of these stages, have replaced the illustrations. Biosafety guidelines for handling blood specimens have been added, in view of the increasing incidence of hepatitis and human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS).

Life cycle and diagnosis

Malaria is transmitted by female *Anopheles* mosquitos. Some 70 of the approximately 420 *Anopheles* species around the world are vectors of human malaria, of which about 40 are of major importance. The mosquito becomes infected with malaria parasites when it bites a person whose blood contains the sexual forms of the parasite (gametocytes). Female macrogametocytes and male microgametocytes ingested in this way become mature gametes in the midgut of the mosquito. Fertilization of a female gamete (macrogamete) by a male gamete (microgamete) results in a motile zygote (ookinete) which migrates to the gut wall and develops into an oocyst. Asexual division inside the oocyst yields as many as 10 000 elongated, spindle-shaped sporozoites, which are released when the oocyst eventually ruptures and migrate via the body cavity to accumulate in the salivary glands of the mosquito. When the infected female *Anopheles* takes its next blood-meal, the sporozoites are inoculated into the bloodstream of the human host. They are carried in the blood to the liver, invading the parenchymal cells where they develop into exo-erythrocytic schizonts.

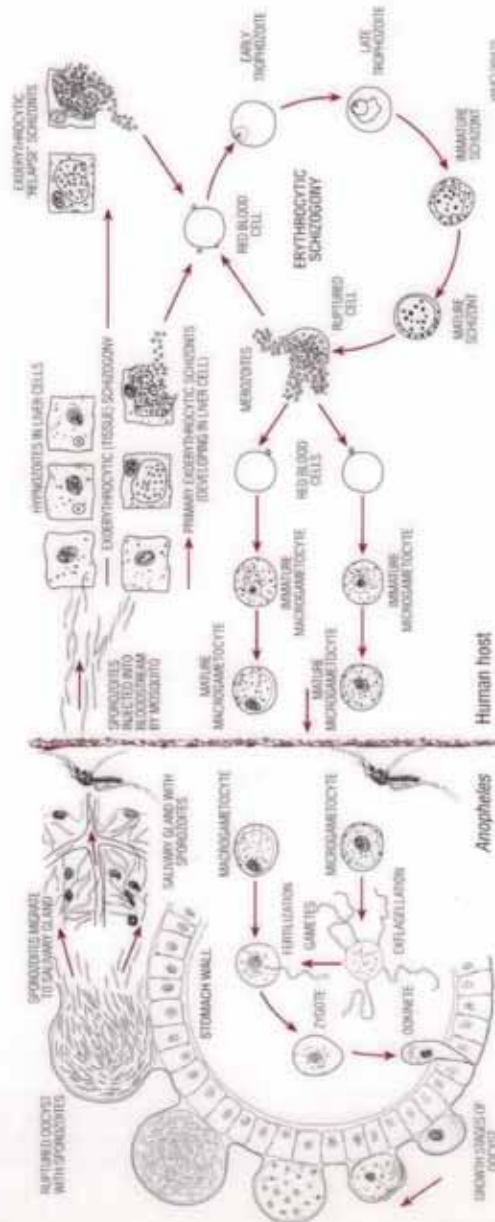
A multiplication phase follows, usually lasting between 5½ and 15 days depending on the species of *Plasmodium*. At the end of this time, the mature schizont bursts, releasing thousands of merozoites (up to 30 000 in *P. falciparum*) into the bloodstream. In *P. vivax* and *P. ovale* malaria, however, some sporozoites do not immediately develop into schizonts; they remain dormant in liver cells for months. Unless these dormant forms — hypnozoites — are destroyed in the liver by specific antimalarial drugs, their later development is responsible for the relapses seen in *P. vivax* and *P. ovale* infections. Since *P. falciparum* and *P. malariae* do not produce hypnozoites, they do not cause relapses, although recrudescence from erythrocytic forms is possible.

In the bloodstream, merozoites invade the red blood cells, where haemoglobin provides nutrition for their development into trophozoites; young trophozoites are known as ring forms because of their shape. Trophozoites develop into schizonts during this erythrocytic (red blood cell) phase and produce malaria pigment as a by-product of their metabolism. Reproduction at this stage is by asexual division (erythrocytic schizogony); after several divisions each mature schizont commonly contains 6–24 merozoites (range 6–40), depending on the *Plasmodium* species. Rupture of the infected red blood cells liberates the merozoites into the bloodstream, where they infect further fresh red cells and begin a new erythrocytic cycle. The repetition of this cycle results in an increasing parasitaemia. After several rounds of erythrocytic schizogony, some of the merozoites differentiate into microgametocytes and macrogametocytes which, when ingested by the female mosquito during a blood-meal, give rise to another cycle of malaria transmission.



Life cycle of malaria

Figure reproduced, with minor amendments, from *Bruce-Chwatt's essential malariaology*, London, Arnold, 1993, with the permission of H.M. Gilles and D.A. Warrell, eds.





Plasmodium falciparum

This species of *Plasmodium* is the most important of the four human species of malaria. Its distribution is concentrated in the tropical and subtropical regions of the world, particularly Africa and Asia. In sub-Saharan Africa it is responsible for almost all recorded malaria and, along with measles, malnutrition, diarrhoea and pneumonia, accounts for most of the deaths in children. In highly malarious areas pregnant women may develop severe anaemia; malaria is an important cause of fetal death. In areas of low transmission all age groups are at risk and, from time to time, epidemics of *P. falciparum* kill thousands of people. Once a patient is infected with *P. falciparum*, it takes 7–27 (average 12) days for the first clinical signs to appear (incubation period). If the patient lacks immunity, infection may quickly develop into an acute form, producing serious damage to the brain (cerebral malaria) and other organs. Cerebral malaria is characterized by coma, which can lead to death. Some patients who recover from cerebral malaria may suffer lifelong neurological sequelae. As there are no hypnozoites in *P. falciparum* infection, only one generation of parasites is produced, but recrudescence may occur up to 1 year later.

In blood films the microscopist usually sees only young trophozoites (ring forms). Gametocytes may also be seen; the more mature trophozoites and schizonts are hidden

away (sequestered) in the organs of the body. In fact, when schizonts are found in blood films, it is usually a sign that infection is heavy and has reached a critical stage. Occasionally, the microscopist will see grains or clumps of pigment (digested haemoglobin), produced by the malaria parasite, within the cytoplasm of white blood cells that have destroyed mature malaria parasites (h). In some films only gametocytes are seen. Infected individuals who have only circulating gametocytes may lack signs and symptoms of the disease, but they can still infect the *Anopheles* mosquitos that bite them. The erythrocytic cycle of *P. falciparum* takes about 48 hours (i.e. it is repeated every third day), although shorter intervals are also possible.

Diagnostic problems: If only a few ring forms are found in blood films, they may be difficult to differentiate from other species of malaria. The presence of large numbers of ring forms in the absence of other stages is strongly suggestive of *P. falciparum* infection; accolé or appliqué forms and multiple infection of red blood cells would further strengthen the diagnosis. Gametocytes are not seen early in the infection, so their absence from blood films should not preclude a diagnosis of *P. falciparum*. Occasionally, only gametocytes may be found in peripheral blood films.

Preparation of buffer solutions for malaria staining

A phosphate buffer solution, correctly balanced to pH 7.2, is essential for Giemsa staining of malaria parasites.

Preparation of a solution for daily use

1. Dissolve 1.0 g of anhydrous disodium hydrogen phosphate (Na_2HPO_4) and 0.7 g of potassium dihydrogen phosphate (KH_2PO_4) in 1000 ml of distilled or deionized water. Filtered rainwater or even tap water may be used if no other is available.
2. Check the pH with a pH meter or colour-based indicator.
3. If the pH is below 7.2, add small quantities of a 2% Na_2HPO_4 solution; if it is above 7.2, add small quantities of a 2% solution of KH_2PO_4 .
4. When balanced to pH 7.2, store the solution in a tightly stoppered bottle, preferably of dark glass, in a cool place away from direct sunlight.

This solution is good for some weeks, but needs to be regularly checked to ensure that growths or moulds do not become established. This may be done by shaking the solution; if cloudy, discard.

Preparation of a concentrated stock solution (useful for field trips or dispatch to distant locations)

1. Dissolve 3.0 g of anhydrous Na_2HPO_4 and 2.1 g of KH_2PO_4 in 25 ml of distilled or deionized water.
2. If the pH is below 7.2, add small quantities of a 2% Na_2HPO_4 solution; if it is above 7.2, add small quantities of a 2% solution of KH_2PO_4 .
3. Store in a dark bottle away from direct sunlight; this solution will remain good for several weeks.
4. To make up a working solution, dilute 1 ml of the concentrate to 20 ml with distilled or deionized water.

Preparation of preweighed packs

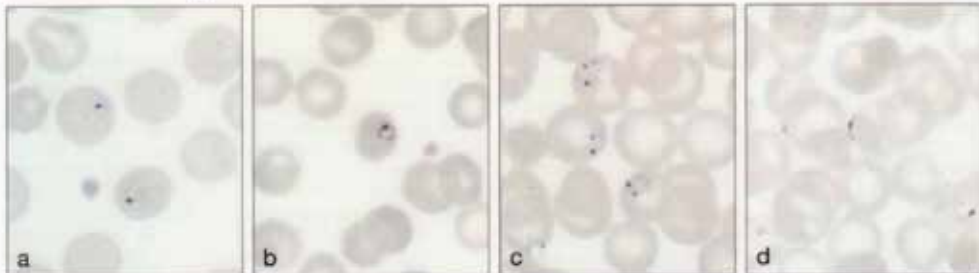
The two phosphate salts can be preweighed and placed together in a clearly labelled, tightly stoppered tube or bottle or in a well sealed plastic bag stored in a screw-capped jar. To make the solution, add the contents to 1 litre of distilled or deionized water and adjust to pH 7.2.

Buffer tablets

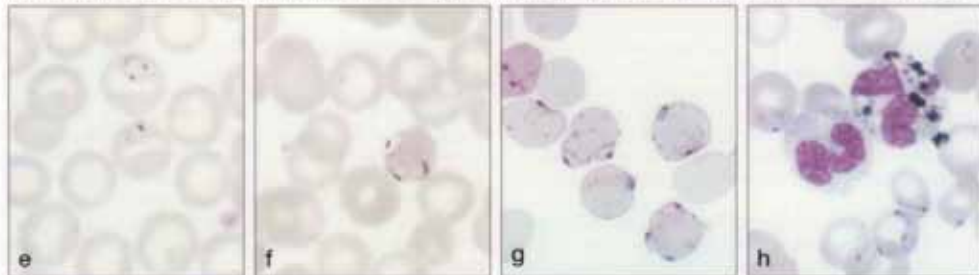
Buffer tablets that produce a solution of pH 7.2 when dissolved are readily available from laboratory suppliers but are rather expensive.

Bench Aids for the diagnosis of malaria infections

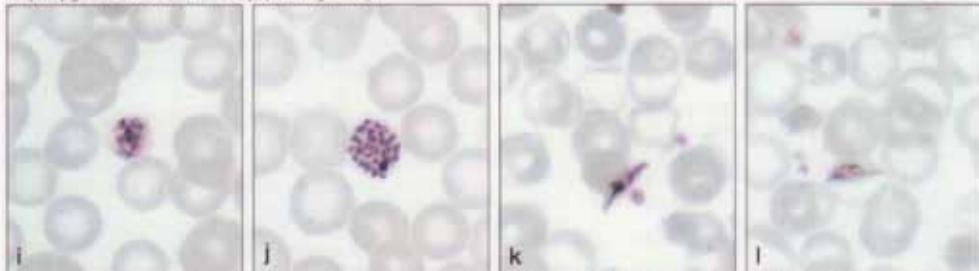
Plate 2

Plasmodium falciparum thin film

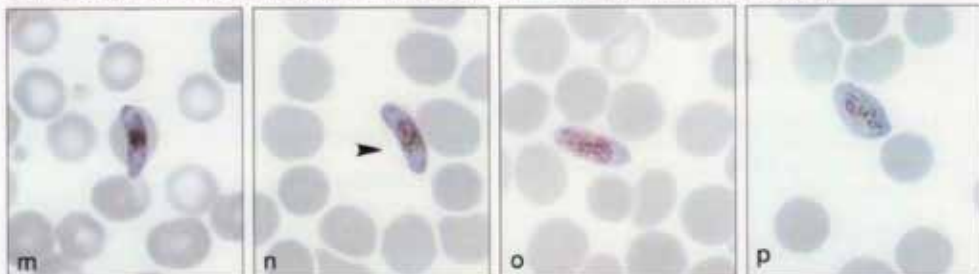
Trophozoites: Merozoites invade red blood cells of all ages. Trophozoites of *P. falciparum* are smaller than those of the other human malarial, tending to have a delicate, thin ring of blue cytoplasm, with a vacuole and a prominent red chromatin dot (a-e). Infected red cells with double chromatin dots (c, e) and multiple invasion of erythrocytes (f, h) are frequent features of this infection. The infected red blood cells are not enlarged. Parasites at the margin of red cells are referred to as accolé or appliqué forms (d); recognition



of these is useful in diagnosis. In some instances, these marginal forms are markedly displaced so that much of the parasite extends beyond the cell margin (f, g). Maurer's clefts (f, g) appear later in *P. falciparum* infection than do Schüffner's dots in *P. vivax* infection. Maurer's clefts are seen in red cells containing older trophozoites and stain best when the pH is alkaline (pH 7.2-7.6). Growing and mature trophozoites are not usually seen in peripheral blood films unless infection is severe and parasitaemia is high. Occasionally lumps of pigment can be seen within the cytoplasm of granulocytes.



Schizonts: These are rarely seen in peripheral blood films except in heavy infections. Mature schizonts are compact, rounded bodies usually containing between 16 and 24 (range 8-40) merozoites (i, j). Pigment in the schizont is usually fused into a single or double mass and may be anywhere in the infected erythrocyte (k, l).



Gametocytes: Initially these are rounded bodies lacking pigment and with no vacuole. As they mature, they become spindle-shaped (k, l) and then develop into characteristic banana- or sausage-shaped bodies with rounded ends (m-o). It is frequently possible to see the red-cell membrane during maturation of the gametocyte (n, arrow). In macrogametocytes, the cytoplasm stains blue and the chromatin is concentrated as a purplish mass (m, n); pigment tends to be more concentrated than in microgametocytes and appears as irregular granules or nodules in the centre of the parasite. The cytoplasm of microgametocytes is often a pinkish purple and the chromatin is more diffuse (o); pigment granules tend to be more scattered than in macrogametocytes. Occasionally, gametocytes may assume bizarre shapes (p).

Bench Aids for the diagnosis of malaria infections



Plate 3

Preparation of thick and thin blood films on the same slide

For routine malaria microscopy, thin and thick blood films are made on the same slide. The thin film is used as a label but, if well prepared, is also available for species confirmation. Examination should be done on a thick film.

The following items are needed for preparation of blood films: clean and wrapped slides; sterile lancets; 70% ethanol and water; absorbent cotton wool; surgical gloves; clean, lint-free cotton cloth; slide box (or cover to exclude flies and dust); record form or register; soft lead pencil; ball-point pen.

1. Holding the patient's left hand, palm upwards, select the third finger from the thumb. (The big toe can be used with infants. The thumb should never be used for adults or children.)

Clean the finger with a piece of cotton wool lightly soaked in 70% ethanol, using firm strokes to remove grease and dirt from the ball of the finger.

Dry the finger with a clean cotton cloth, using firm strokes to stimulate blood circulation.

2. Puncture the ball of the finger with a sterile lancet, using a quick rolling action.

Apply gentle pressure to the finger to express the first drop of blood and wipe it away with a dry piece of cotton wool. Make sure that no strands of cotton remain on the finger to be later mixed with the blood.

3. Working quickly and handling clean slides only by the edges, collect the blood as follows.

Apply gentle pressure to the finger and collect a single small drop of blood, about this size ●, on the middle of the slide. This is for the thin film.

Apply further pressure to express more blood and collect two or three larger drops, about this size ●, on the slide, about 1 cm from the drop intended for the thin film (see illustration). This is for the thick film.

Wipe the remaining blood away from the finger with a piece of cotton wool.

4. **Thin film.** Using a second clean slide as a "spreader" and, with the slide with the blood drops resting on a flat, firm surface, touch the small drop with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide, keeping the spreader at an angle of 45°. Make sure that the spreader is in even contact with the surface of the slide all the time the blood is being spread.

5. **Thick film.** Always handle slides by the edges or by a corner to make the thick film as follows.

Using the corner of the spreader, quickly join the drops of blood and spread them to make an even, thick film. The blood should not be excessively stirred but can be spread in circular or rectangular form with 3 to 6 movements. The circular thick films should be about 1 cm (1/2 inch) in diameter.

6. Label the dry thin film with a soft lead pencil by writing across the thicker portion of the thin film the patient's name or number and the date. Do not use a ball-point pen for labelling the slide. Allow the thick film to dry with the slide in a flat, level position, protected from flies, dust and extreme heat.

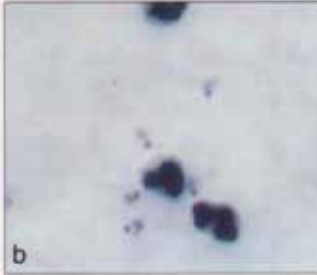
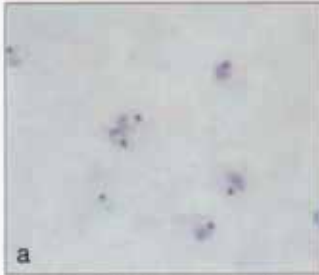
7. Wrap the dry slide in the patient's record form and dispatch it to the laboratory as soon as possible.

8. The second slide used for spreading the blood films may now be used for the next patient and another clean slide from the pack will be used as a spreader.

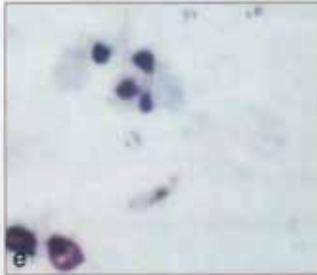
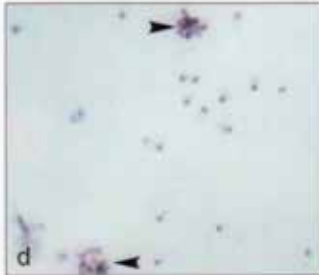


Bench Aids for the diagnosis of malaria infections

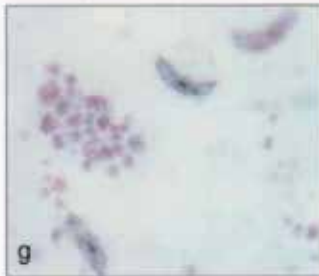
Plate 3

***Plasmodium falciparum* thick film**

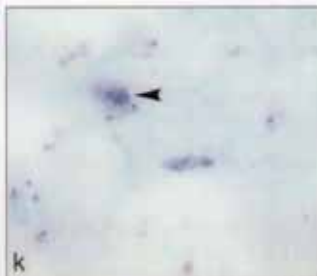
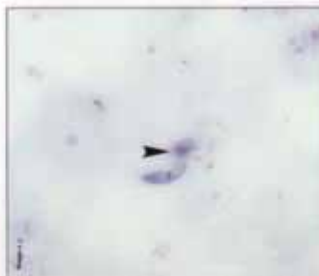
Ring forms (a–c) are usually small, often numerous, with delicate, scanty cytoplasm. Ring and comma forms (distorted ring forms) are common but older ring forms may have more cytoplasm (a). Ring forms with double chromatin dots are common. The presence of large numbers of ring forms in the absence of other morphological stages is generally diagnostic for this species (c).



In heavy infections, small, compact schizonts (d, arrows), usually containing between 16 and 24 (range 8–40) merozoites, may be found clustered around a small, dark mass of pigment (d); large numbers of ring forms are also present. Ring forms may be found with gametocytes (e) and, in some infections, gametocytes may be found in a field in the absence of ring forms (f).



Typical banana- or sausage-shaped gametocytes (g, h), sometimes referred to as crescents, are easy to identify. However, when damaged or altered in the process of making thick films, gametocytes (i, arrows), in the absence of ring forms, may be more difficult to recognize.



In mixed infections of *P. falciparum* and *P. vivax* (j–l), a typical gametocyte and numerous small ring forms of *P. falciparum* are visible, along with a *P. vivax* trophozoite (j, arrow). In (k) ring forms and a gametocyte are present with a large trophozoite of *P. vivax* (arrow). In (l) a gametocyte is visible along with a prominent trophozoite of *P. vivax* (arrow).

Bench Aids for the diagnosis of malaria infections



Plate 4

Plasmodium vivax

Plasmodium vivax occurs throughout the tropics and subtropics and is also the predominant species in temperate climates. It is very rare in West Africa. The incubation period is typically about 13–17 days, although some strains may have prolonged incubation periods of up to 6–12 months. An important feature of this species is the presence and persistence of exoerythrocytic stages (hypnozoites) in the liver, which may produce relapses of infection repeatedly over a period of many years. The erythrocytic cycle usually requires about 48 hours and all the characteristic morphological stages of trophozoites, schizonts and gametocytes may be found in the peripheral blood.

Diagnostic problems: If only a few ring forms are found in blood films, diagnosis is very difficult. It may be necessary to make an extended examination of the slide for other stages and for Schüffner's stippling. Examination of thick blood films may be necessary to demonstrate additional parasites. It should be remembered that, in thick films, the periphery of the film may be poorly lysed and "ghosts" of red blood cells may demonstrate organisms as well as signs of stippling (Plate 5, f).

Biosafety in the handling of blood specimens from patients

The hazards that technical staff encounter in laboratories are widely recognized. All laboratories should follow national guidelines on safety or they may have guidelines that have been developed locally. A number of guidelines are also available from WHO; these include *Safety in health-care laboratories*, *Guidelines on sterilization and disinfection methods effective against human immunodeficiency virus (HIV)*, 2nd ed., *Biosafety guidelines for diagnostic and research laboratories working with HIV* and *Preventing HIV transmission in health facilities* (see "Further reading" section).

All blood samples must be considered as potentially infectious. Two of the more dangerous bloodborne diseases are hepatitis (caused mainly by hepatitis viruses B and C) and HIV/AIDS. When blood samples are collected for diagnosis of malaria, biosafety guidelines must be followed.

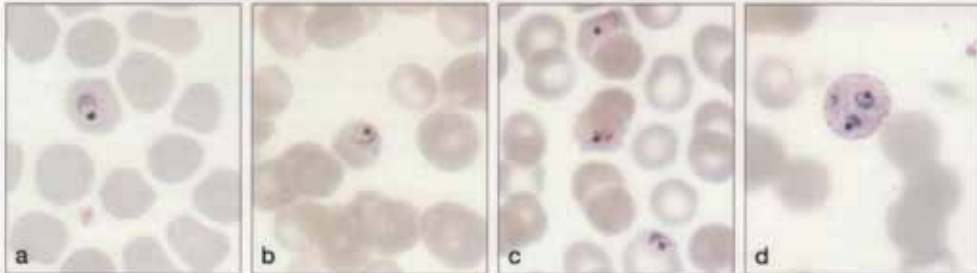
The major hazard to laboratory workers taking blood specimens is contamination of the hands and the mucous membranes of the eyes, nose and mouth by infectious blood. Such contamination occurs as a result of penetrating injuries caused by sharp objects, and the spilling or splashing of specimens. The guidelines given here outline practices and procedures designed to keep such accidents to a minimum.

1. All laboratory workers must be adequately trained, both in the duties they perform and in all aspects of laboratory work.
2. Standard operating procedures should be clearly written, which cover all steps in the procedures to be carried out.
3. Wear a laboratory gown, smock or uniform when in the laboratory. Remove this protective clothing before leaving the laboratory.

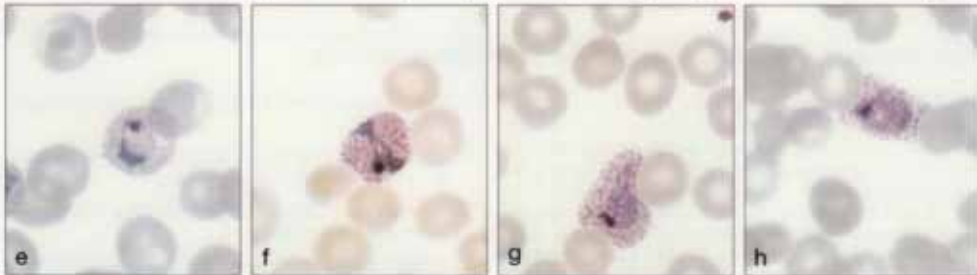
4. Wear gloves when taking and handling blood specimens.
5. Do not touch your eyes, nose or other exposed membranes or the skin with gloved hands.
6. Do not leave the workplace or walk around the laboratory wearing gloves.
7. Discard gloves whenever they are thought to have become contaminated, wash your hands, and put on new gloves.
8. Wash your hands with soap and water immediately after any contamination and after work is completed. If gloves are worn, wash your hands with soap and water after removing the gloves.
9. Puncture wounds, cuts and skin contaminated by spills or splashes of blood should be thoroughly washed with soap and water. Bleeding from the wound should be encouraged.
10. All spills, accidents and overt or potential exposure to infectious specimens should be reported immediately to the laboratory supervisor and appropriate action should be taken to prevent further occurrences.
11. Place used lancets in a puncture-resistant container.
12. Disinfect work surfaces when procedures are completed and at the end of each working day. An effective all-purpose disinfectant is a hypochlorite solution with a concentration that provides 0.1% available chlorine (1 g/litre).
13. Do not eat, drink or smoke in the laboratory.
14. Access to the laboratory must be restricted to authorized personnel only.

Bench Aids for the diagnosis of malaria infections

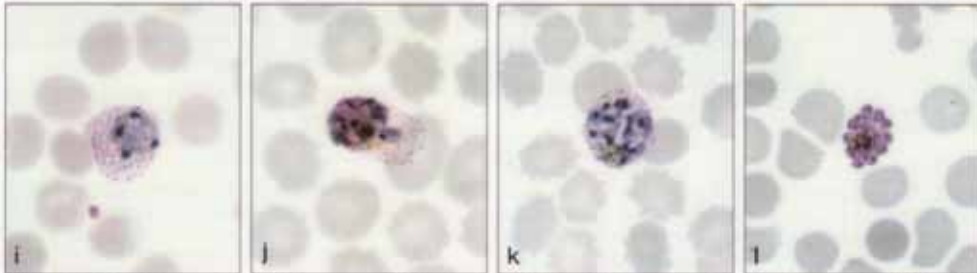
Plate 4

***Plasmodium vivax* thin film**

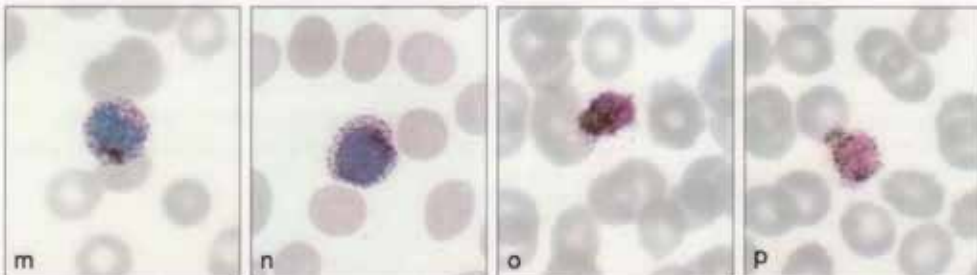
Trophozoites: Merozoites typically invade young erythrocytes. The infected red blood cells become enlarged, often by more than about 50% (about the size of a white blood cell, and may vary from round to oval. Very young trophozoites (ring forms) typically measure about one-third the diameter of the red blood cell; they are composed of a prominent red chromatin dot and a fine circle of blue cytoplasm (a–d). Occasionally there may be two chromatin dots. Young growing trophozoites have an increased mass of cytoplasm



and usually an irregular, amoeboid appearance (e). Older trophozoites become very large and markedly amoeboid and can fill the red blood cell (f–h). The chromatin mass is large and compact; grains of pigment are scattered throughout the cytoplasm and a conspicuous vacuole is almost always present (h). Mature trophozoites may be difficult to differentiate from gametocytes (h, m). In properly stained blood films, Schöffer's dots may be seen in red blood cells containing young trophozoites (e–g) and, rarely, earlier (d).



Schizonts: Early in its development, the schizont is large and amoeboid. The chromatin divides into small irregular masses, ultimately forming between 14 and 20 (range 12–24) merozoites (i–l). In the mature schizont, one or two clumps of pigment may be centred in the cluster of merozoites (l). Each merozoite is composed of a dot of chromatin surrounded by a small mass of cytoplasm (l). The mature schizont usually fills the enlarged red blood cell (k, l). Schöffer's stippling is usually evident in the infected cell (k).



Gametocytes: The parasites are usually round to oval and regular in outline (m–p). Macrogametocytes (m, n) are typically large and blue and have a small, eccentric, compact chromatin mass. Brown pigment is scattered throughout the cytoplasm and vacuoles are absent. The mature parasite nearly fills the enlarged red blood cell (m, n); this stage is often difficult to distinguish from a mature trophozoite (h). Microgametocytes (o, p) have a large, diffuse mass of pink-staining chromatin and light blue to pink or lavender cytoplasm containing scattered granules of dark pigment.



Staining blood films for malaria parasites

The use of Giemsa stain is the recommended and most reliable procedure for staining thick and thin blood films to demonstrate malaria parasites. It is available as a commercially prepared solution or in powder form; however, since the quality of the stain may vary it should be acquired from a reputable manufacturer and each batch of prepared stain should be evaluated before being used to stain large numbers of slides. Further information on stain and buffer solution preparation and staining procedures is provided in the WHO publication *Basic laboratory techniques in medical parasitology* (see "Further reading" section).

Preparation of stock solution of Giemsa stain

Giemsa stain formula: Giemsa powder, 3.8 g; methanol, 250 ml; glycerol, 250 ml.

Giemsa stain preparation

1. Put 50 solid glass beads in a dark bottle. If a dark bottle is not available, use a chemically clean, dry, clear glass or polyethylene bottle of suitable size. Pour in the measured amount of methanol and add the stain powder.
2. Tightly stopper the bottle. Allow the stain powder to sink slowly through the methanol until it settles to the bottom. Shake the bottle with a circular motion for 2–3 minutes.
3. Add the measured amount of glycerol and repeat the shaking process. Continue to shake the bottle for 2–3 minutes at half-hourly intervals for at least six times.
4. Leave the bottle for 2–3 days, shaking it 3–4 times each day until the stain is thoroughly mixed. This is the stock solution. Keep some of this stock solution in a small bottle for routine use to avoid contamination of the stock solution.

Each newly prepared batch of stock solution should be properly labelled, including date of preparation, and should be tested for optimum stain dilution and staining time. Always keep the bottle, tightly stoppered, in a cool place, away from direct sunlight. Clear glass bottles should be covered with thick dark paper to exclude the light.

Giemsa staining technique — regular method

Ideally, for optimum staining, thick and thin films should be made on separate slides. This is often not possible and thick and thin films are generally made on the same slide. When this is done, good-quality staining of the thick film is of primary importance. Allow the thick film to dry in a flat, level position, protected from flies, dust and extreme heat. It is important to note that, in tropical countries, autofixation may occur. This is a process by which blood films gradually become fixed through exposure to the atmosphere. In tropical conditions, this process may become well advanced with films stored for only a few days. When storing thick films for later staining it is particularly important to keep them in a dry atmosphere; a desiccator may be used for this purpose.

Fixation procedure

Once the thin film has dried, fix it by adding a few drops of methanol, or by dipping it into a container of methanol for a few seconds. With prolonged fixation it may be difficult to demonstrate Schüffner's dots and Maurer's clefts. Allow the thin film to dry thoroughly by evaporation. Exposure of the thick film to methanol or methanol vapour should be avoided. If the methanol does fix the thick film, dehaemoglobinization will not be possible.

Staining procedure (for 20 or more slides)

Place the slides in a staining trough. Prepare a 3% Giemsa solution in buffered distilled or deionized water, pH 7.2, in sufficient quantity to fill the trough and cover the slides. Stain for 30–45 minutes, out of sunlight. Pour clean water gently into the trough to float off the iridescent scum on the surface of the stain. Rinse rapidly in clean water. Remove the slides one by one and place them, film side downwards, in a slide rack to drain and dry, making sure that the film does not touch the rack.

Staining results

On the thick film, the background should be clean and free from debris; leukocyte nuclei should be a deep, rich purple, and the malaria parasites should have deep red chromatin and pale purplish blue cytoplasm. At the periphery of the thick film, erythrocytes are not lysed and Schüffner's stippling may be apparent in *P. vivax* and *P. ovale* infections.

Giemsa staining technique — rapid method

This is a satisfactory procedure but uses much more stain than the regular method.

Fixation procedure

Once the thin film has dried, fix it by adding a few drops of methanol or by dipping the film in a container of methanol for a few seconds. To permit dehaemoglobinization, the thick film should not be fixed; exposure of the film to methanol or methanol vapour should therefore be avoided. Allow to dry thoroughly by evaporation.

Staining procedure

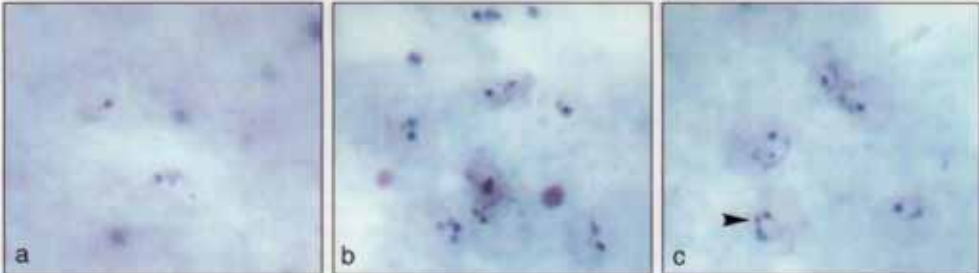
Prepare a 10% Giemsa solution in buffered distilled or deionized water, pH 7.2; if a small quantity is being used, 3 drops of stain per ml of buffered water will give the correct concentration of Giemsa solution. One slide requires about 3 ml of stain solution. Gently pour the stain onto the slide or use a pipette. Alternatively, the slide can be placed face-down on a concave staining plate and stain introduced underneath the slide. Stain for 5–10 minutes. Gently flush the stain off the slide by adding drops of clean water; do not tip off the stain and then wash, as this will leave a deposit of scum over the film. Place slides, film side downwards, in a slide rack to drain and dry, making sure the film does not touch the rack.

Staining results

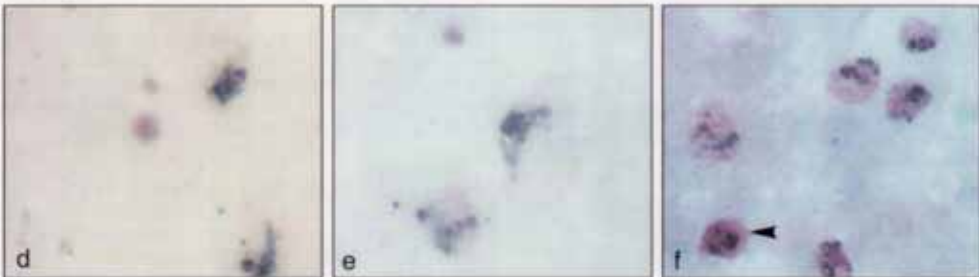
These are the same as are obtained in the regular method of Giemsa staining.

Bench Aids for the diagnosis of malaria infections

Plate 5

Plasmodium vivax thick film

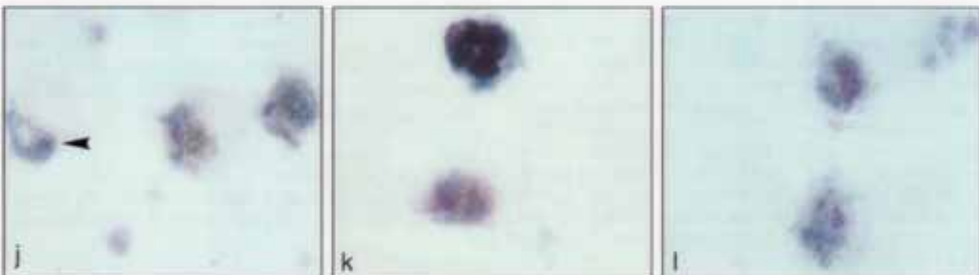
Typical ring forms (a–c), and one with a double chromatin dot (c, arrow), vary somewhat in size and have prominent red chromatin dots with varying amounts of light blue chromatin. The ring forms are typically larger than those of *P. falciparum*, often without a complete circle of blue cytoplasm. Young trophozoites (b, c) are visible in “ghosts” of erythrocytes.



Trophozoites (d–f) of this species may vary in both size and the numbers in which they are present. The cytoplasm is darker and thicker (d, e) than that seen in the “ghosts” of red blood cells containing amoeboid organisms with irregular and fragmented cytoplasm (f). Schüffner's stippling is evident in several of the infected erythrocytes (f). A gametocyte is also visible (f, arrow). Large trophozoites are often dense, compact and darkly staining and contain scattered pigment; they can be confused with macrogametocytes.



Immature (g, h) and mature (i) schizonts are usually large and present in small to moderate numbers. Mature schizonts usually contain between 16 and 24 merozoites and have a loose mass of pigment. Individual chromatin masses are irregular in shape and often quite large in immature schizonts. Immature schizonts may be confused with those of *P. malariae*.



Gametocytes (j–l) of *P. vivax* are usually larger than those of other species. Mature forms are usually large and round; pigment granules are fine and dispersed throughout the non-vacuolated cytoplasm. Chromatin masses are dense and may or may not be well defined. Differentiation of gametocytes, especially immature forms, from mature trophozoites (j, arrow) is often difficult.



Plasmodium ovale

The global distribution of *P. ovale* is more limited than that of other *Plasmodium* species; it is found mainly in tropical Africa where *P. vivax* is rare. It is also present in New Guinea and the Philippines, with occasional reported occurrences in other parts of south-east Asia. Like *P. vivax*, *P. ovale* has an incubation period of approximately 16–18 days, an asexual cycle of about 50 hours and persistent exoerythrocytic stages (hypnozoites) in the liver which can produce relapses of infection. As in *P. vivax* infection, red blood cells typically show Schüffner's stippling; generally it develops much earlier in young trophozoites and the granules tend to be more prominent. All erythrocytic stages are usually present in the bloodstream.

Diagnostic problems: This species is the most difficult to diagnose because of morphological similarities to both *P. vivax* and *P. malariae*. Schüffner's stippling is similar to that seen in *P. vivax*. Oval or elongated infected red blood cells, which are very common in *P. ovale* infections, are occasionally seen in *P. vivax* infections. A history of the patient's residence and travel is important in establishing the diagnosis.

Rapid staining of blood films for malaria parasites

A rapid staining technique for thick blood films which can produce excellent results involves the use of Field's stain; this procedure is especially useful when single thick films are submitted for evaluation. Further information on stain and buffer solution preparation and staining procedures is provided in the WHO publication *Basic laboratory methods in medical parasitology* (see "Further reading" section).

Preparation of Field's stain

This stain is useful for rapid detection of malaria parasites; it is usually used for thick blood films. Schüffner's dots are not always stained with this procedure. Both Field's stain A and Field's stain B must be made up for staining slides, as both are used in the procedure.

Field's stain A — stock solution

Field's stain A stock solution may be prepared in two different ways, depending on whether prepared powders or original stains and chemicals are being used.

Preparation from prepared powders:

1. Add 5.9 g of Field's stain A powder to 600 ml of hot (approx. 60 °C) distilled water.
2. Mix until dissolved.
3. Filter when cool.

Preparation from original stains and chemicals:

1. Dissolve 10.0 g of anhydrous disodium hydrogen phosphate (Na_2HPO_4) and 12.5 g of potassium dihydrogen phosphate (KH_2PO_4) in 1000 ml of distilled water.
2. Pour half of this solution into a 1-litre bottle containing a few glass beads. Add 1.6 g of methylene blue (medicinal) and 1.0 g of azur I and mix well.
3. Add the remainder of the phosphate solution.
4. Mix well and filter.

Field's stain B — stock solution

Field's stain B stock solution may be prepared in two different ways, depending on whether prepared powders or original stains and chemicals are being used.

Preparation from prepared powders:

1. Add 4.8 g of Field's stain B powder to 600 ml of hot (approx. 60 °C) distilled water.
2. Mix until dissolved.
3. Filter when cool.

Preparation from original stains and chemicals:

1. Dissolve 10.0 g of anhydrous disodium hydrogen phosphate (Na_2HPO_4) and 12.5 g of potassium dihydrogen phosphate (KH_2PO_4) in 1000 ml of distilled water.
2. Add 2.0 g of eosin (yellow, water-soluble).
3. Mix until dissolved.
4. Filter.

Staining with Field's stain (thick and thin films)

Procedure for staining thick films

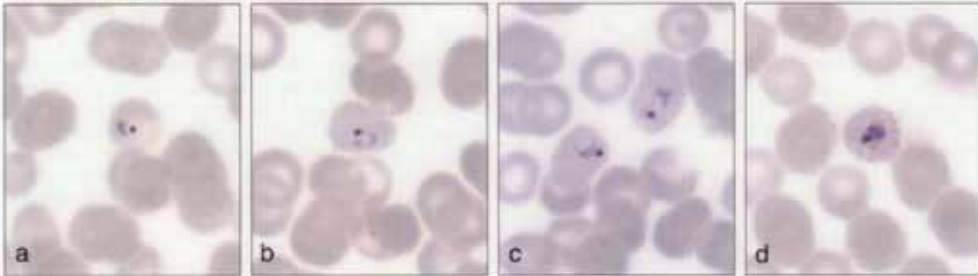
1. Dip unfixed film into a jar containing Field's stain A solution for 3 seconds.
2. Wash gently by dipping (once) into a jar of clean water.
3. Dip slide into a jar containing Field's stain B solution for 3 seconds.
4. Wash slide gently as in step 2.
5. Place slide upright in a draining rack to air-dry.

Procedure for staining thin films

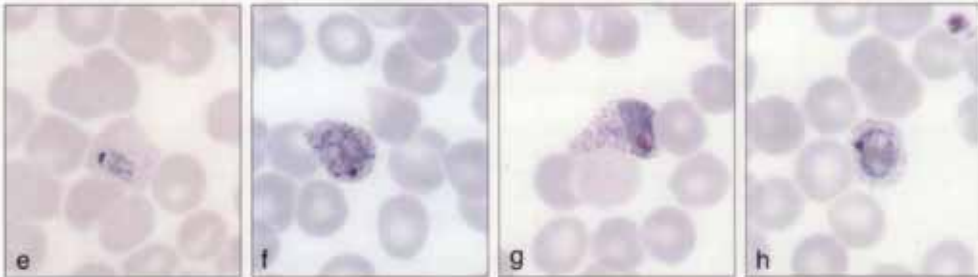
1. Fix film in methanol for 1 minute.
2. Wash off methanol with water.
3. Using a pipette, cover the film with diluted Field's stain B (1 part by volume of stock stain solution plus 4 volumes of distilled water buffered at pH 7.2).
4. Immediately add an equal volume of Field's stain A solution and mix well by tilting the slide.
5. Allow to stain for 1 minute.
6. Wash off stain with clean water.
7. Place slide upright in a draining rack to air-dry.

Bench Aids for the diagnosis of malaria infections

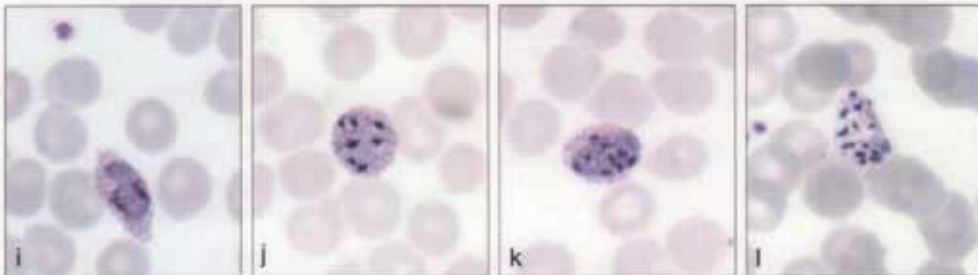
Plate 6

***Plasmodium ovale* thin film**

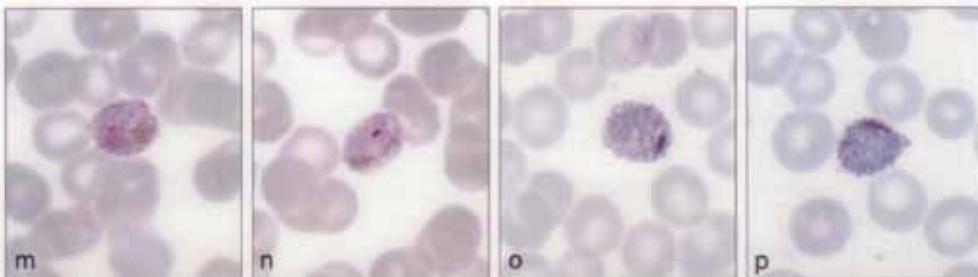
Trophozoites: Merzoites, like those of *P. vivax*, invade young erythrocytes; the ring stage resembles that of the other human malaria species (a, b). Infected red blood cells may be round but are often oval in shape and may or may not have an irregular margin (c, d). In young trophozoites, the mass of chromatin may be large and irregular in shape



and may show Schüffner's stippling (e–f). More mature trophozoites are often in slightly enlarged red cells (f), which may be oddly shaped with fringed (fimbriated) margins (g, h). The trophozoites tend to be less amoeboid in appearance than those of *P. vivax*.



Schizonts: Developing (i–k) and mature (l) schizonts are frequently found in oval-shaped cells, many with irregular margins (i). Erythrocytes may be only slightly enlarged. Mature schizonts usually have 6–12 merozoites, but occasionally up to 18 (k, l). Clumps of pigment are often found at the centre of the cluster of merozoites. Schüffner's stippling is usually prominent.



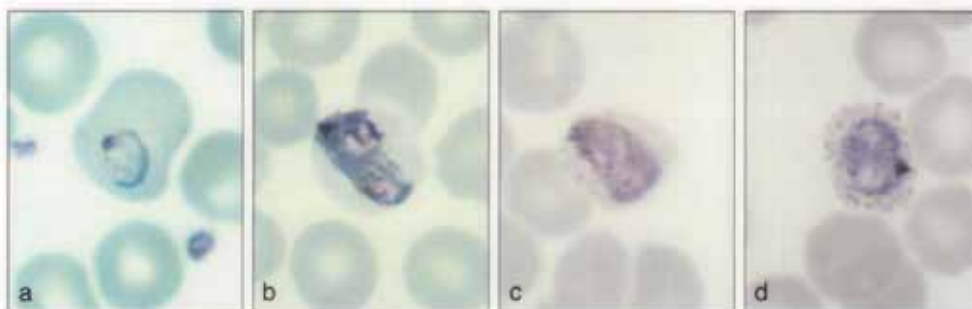
Gametocytes: Mature gametocytes typically fill the host red blood cells, which may be round or oval with sometimes irregular margins (m–p). Pigment granules are scattered throughout the cytoplasm or, in microgametocytes (m, n), concentrated towards the periphery of the organisms. The gametocytes of *P. ovale* are often difficult to distinguish from those of *P. vivax*. Microgametocytes (m, n) are usually smaller than macrogametocytes and have diffuse pink chromatin. In macrogametocytes (o, p), chromatin is compact and usually dark red. Schüffner's stippling is prominent in the infected red cells.



Effect of pH on Giemsa staining of malaria parasites

These four figures of *P. vivax* illustrate the effect of pH on Giemsa staining of malaria parasites and blood elements. In (a), note the markedly greenish blue staining of the erythrocytes at a pH of 7.6. A trophozoite is recognizable but the Schüffner's stippling in the erythrocyte cytoplasm is faint. At a pH of 7.4 (b), the erythrocyte containing a

trophozoite has a light greenish blue tinge and again Schüffner's stippling in the cytoplasm is pale and barely recognizable. At the more ideal pH values of 6.8 (c) and 7.0 (d), the red blood cells have a pinkish appearance, the trophozoites stain well and Schüffner's stippling is prominent.



Routine examination of blood films for malaria parasites

Examining thin films

Routine examination of thin films is not recommended as it takes at least three times as long to examine the equivalent amount of blood in a thin film as it does in a thick film. The exceptions to this rule are for poorly prepared thick films or when confirmation of species identification is necessary.

Procedure

It is usually in the distal third of the blood film that cells: (i) are most evenly distributed; (ii) are in a single layer; and (iii) have minimum distortion. This is where the microscopist should devote most of his or her attention. Place the slide on the mechanical stage, and position the x100 oil-immersion objective as shown by the letter X in Fig. 1. Add a drop of immersion oil to the slide and lower the objective until it touches the oil. Examine the blood film following the pattern of movement shown.

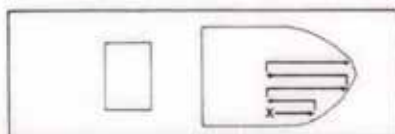


Fig. 1

Examining thick films

Procedure

Routinely, it is thick films that are examined. Provided that they have been well made and stained before autofixation could occur, there should be no problems in identifying the species of malaria parasites. Place the slide on the mechanical stage, and position the x100 oil-immersion objective as shown by the letter X in Fig. 2. Add a drop of immersion oil to the slide and lower the objective until it touches the oil. Examine 100 fields, following the pattern of movement shown. If parasites are found, examine another 100 fields before confirming the identification of species present; this allows for possible detection of a mixed infection. To assist in examination, use a hand tally counter to count fields. Record findings on the appropriate record form. A parasite count may be included.

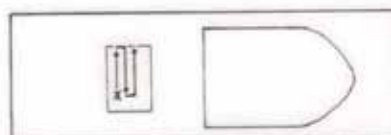
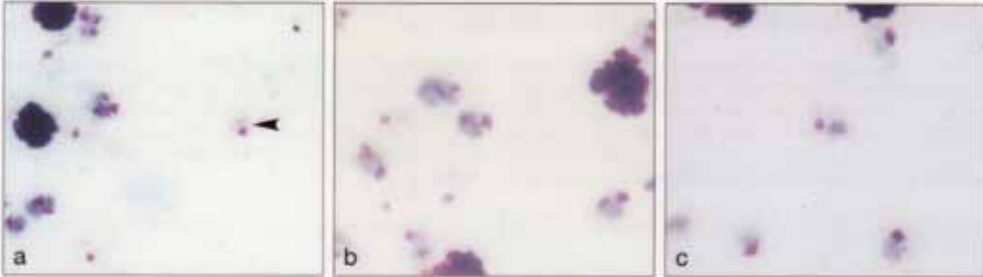


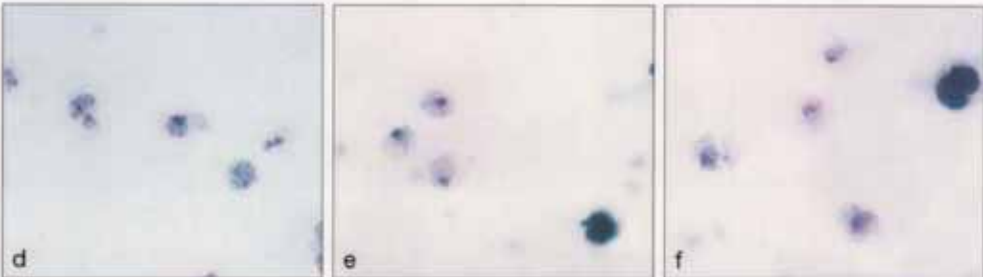
Fig. 2

Bench Aids for the diagnosis of malaria infections

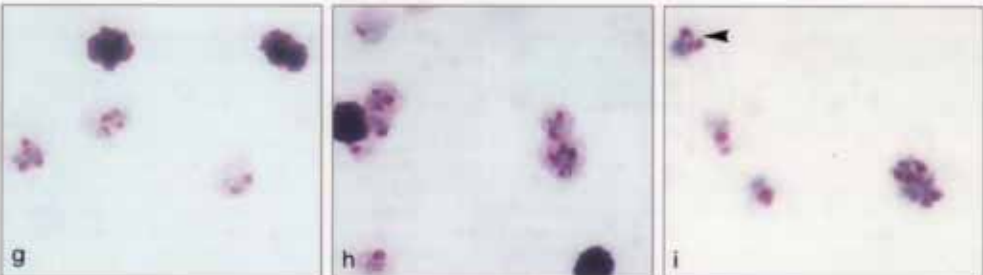
Plate 7

***Plasmodium ovale* thick film**

Ring forms and young trophozoites (a–c) of *P. ovale* are similar to those of *P. vivax*. A typical ring form with a prominent chromatin dot and wispy of blue cytoplasm is present (a, arrow), but most of the forms (a–c) are young trophozoites with a prominent mass of blue.



Compact, young trophozoites are present (d), but many of the trophozoites seen (e, f) are mature and have a somewhat amoeboid form. Stippling is frequently pronounced and appears as a pinkish "cloud" around the parasite (d–f).



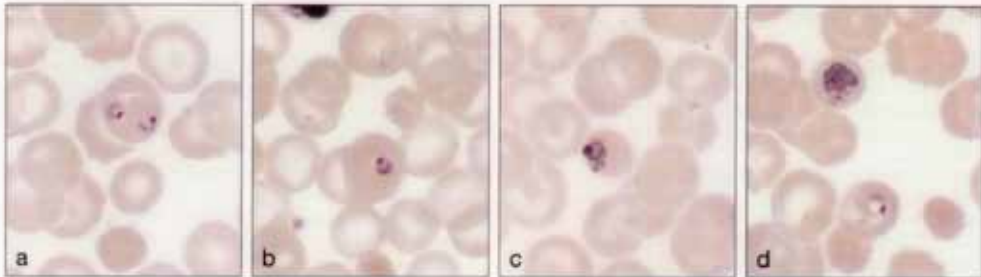
Schizonts of *P. ovale* are usually few in number and similar in size to those of *P. malariae*; immature schizonts with little pigment are evident (g). Mature schizonts contain 6–12 merozoites and pigment is usually seen as a concentrated mass (h). A mature schizont with a compact mass of pigment can be distinguished, along with two trophozoites and an immature schizont (i, arrow).



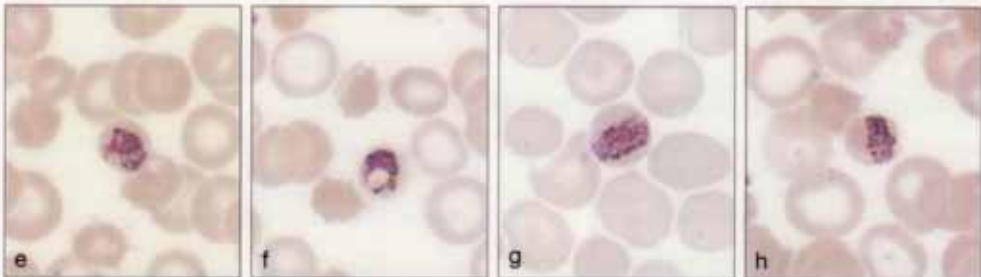
Immature and mature gametocytes of *P. ovale* may be difficult to distinguish from mature trophozoites. In addition, gametocytes of this species are similar in size and morphology to those of *P. vivax*. Two rounded gametocytes with considerable coarse pigment and a trophozoite (j, arrow) illustrate how difficult it is to distinguish between these stages. Two trophozoites (right) and two gametocytes (left) are visible (k), and also a single gametocyte with pigment (l).

Bench Aids for the diagnosis of malaria infections

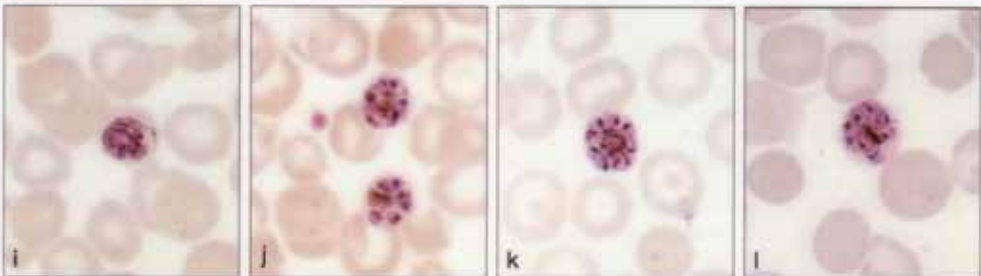
Plate 8

Plasmodium malariae thin film

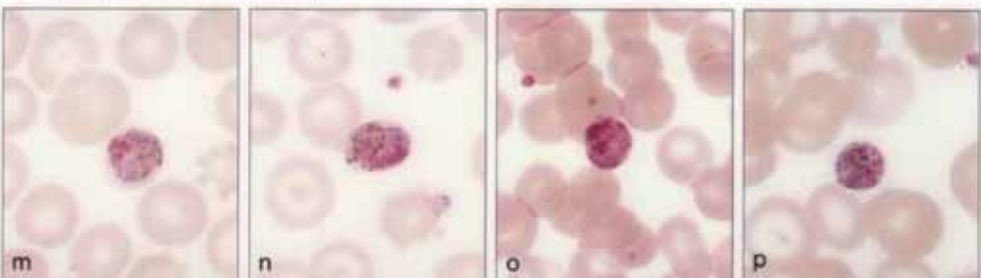
Trophozoites: Merzoites invade mature red blood cells. Ring forms are fairly large and the chromatin dot may be peripheral (a–d). Occasionally the chromatin dot may be located in the centre of the vacuole. Double chromatin dots and invasion of red cells by more than one organism (a) are uncommon. Trophozoites grow slowly and often they may stretch



across the equator of the red cell to produce the typical band forms associated with this species (g, h). Other trophozoites may exhibit a large vacuole surrounded by dense cytoplasm and an elongated mass of chromatin, producing a basket-like appearance (f). Mature trophozoites have considerable coarse, brown pigment and almost fill the erythrocyte (d, e).



Schizonts: Immature schizonts show fewer divisions of chromatin into irregular sizes and shapes (i). Mature schizonts usually have 8 or 10 merozoites (range 8–12), which are characteristically arranged in a rosette formation surrounding a mass of brown pigment (k). However, many schizonts, which usually fill the red blood cell (j, k), may have the pigment clumped at the periphery of the organism (l).



Gametocytes: It is difficult to differentiate between gametocytes and mature trophozoites in this species. Macrogametocytes (m, n) have a bluer cytoplasm and the chromatin is smaller, redder and more compact than that seen in microgametocytes. The cytoplasm of the microgametocyte is a light bluish pink (p) and the chromatin is diffuse, pinkish blue (o). Brown pigment is conspicuous with granules scattered throughout the cytoplasm (m–p).

Bench Aids for the diagnosis of malaria infections



Plate 9

Common faults in making blood films

A number of faults are common in making blood films. These can affect the labelling, the staining or the examination, and sometimes more than one of these.

Badly positioned blood films

Care should be taken that the blood films are correctly sited on the slide. If they are not, it may be difficult to examine the thick film. Also, portions of the films may even be rubbed off during the staining or drying process.

Too much blood

After staining of films with too much blood, the background to the thick film will be too blue. There will be too many white blood cells per thick film field, and these could obscure or cover up any malaria parasites that are present. If the thin film is too thick, red blood cells will be on top of one another and it will be impossible to examine them properly after fixation.



Too little blood

If too little blood is used to make the film, there will not be enough white cells in the thick film field and you will not examine enough blood in the standard examination. The thin film may be too small to use as a label.



Blood films spread on a greasy slide

The blood films will spread unevenly on a greasy slide, which makes examination very difficult. Some of the thick film will probably come off the slide during the staining process.



Edge of spreader slide chipped

When the edge of the spreader slide is chipped, the thin film spreads unevenly, is streaky and has many "tails". The spreading of the thick film may also be affected.



Thin film too big, thick film in the wrong place

If the thin film is too large, the thick film will be out of place and may be so near the edge of the slide that it cannot be seen through the microscope. During staining or drying, portions of the thick film will probably be scraped off by the edges of the staining trough or drying rack. It may be very difficult, or impossible, to position the thick film on the microscope stage so that it can be examined.



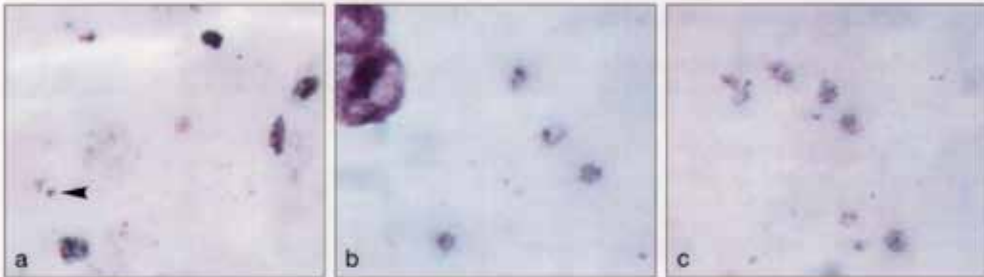
Other common faults

Other faults that occur commonly in the preparation of blood films include the following:

- Flies, cockroaches or ants eat the dry blood and damage the films.
- Blood films are made on badly scratched slides or on slides with "frozen" or iridescent surfaces.
- The thick film is allowed to dry unevenly.
- Autofixation of the thick film occurs with the passage of time or through exposure to heat, and staining then becomes difficult or unsatisfactory.
- Slides are wrapped together before all the thick films are properly dried, and the slides stick to one another.

Bench Aids for the diagnosis of malaria infections

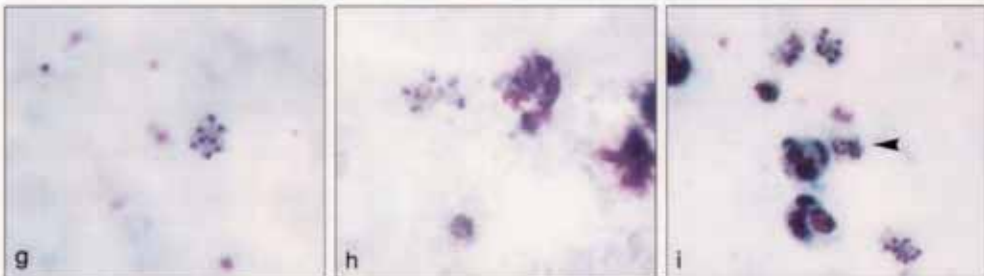
Plate 9

***Plasmodium malariae* thick film**

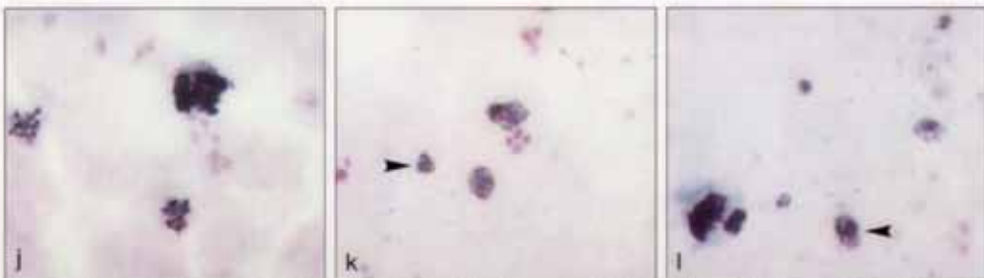
Ring forms tend to be small and few in number and have large chromatin dots and a small amount of cytoplasm, often without a vacuole (a, b). Ring forms may (a) lack a complete circle of cytoplasm, but in a field containing several trophozoites a complete ring form (b) can be seen. Early trophozoites (c) may lack a vacuole. Pigment forms early in this species and is present as dark, coarse grains (b, c).



Growing trophozoites (d, e) vary in shape. Three small, rounded trophozoites are present (e, arrows), along with a growing trophozoite (right). A schizont (f, arrow) containing 8 merozoites and a compact mass of pigment is visible, along with a few rounded trophozoites and two leukocytes.



Mature schizonts, containing 8 merozoites, are visible (g-h); small, dark, concentrated masses of pigment occur in each of the schizonts. In (h), the schizont and a rounded trophozoite are present. An immature schizont (i, arrow) is seen in the same field as three mature schizonts (i).



Two mature schizonts and a leukocyte are visible (j). Two gametocytes are larger (k) than the small, rounded trophozoites (k, arrow). A single gametocyte (l, arrow) is seen with small ring forms and growing trophozoites. Gametocytes may be difficult to distinguish from mature trophozoites; coarse pigment grains are often peripherally distributed in gametocytes but not in trophozoites.

Bench Aids for the diagnosis of malaria infections

Plate 10

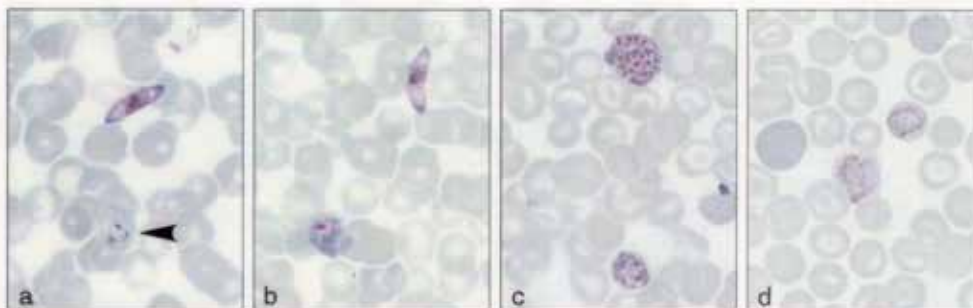
Mixed infections

Infections with more than one species of malaria parasites may be encountered in many areas where two or more species are endemic. These infections may be overlooked by microscopists for a variety of reasons. If only thin blood films are made for diagnosis, as happens in many laboratories, low-density parasitaemias of one or the other species may be masked by the predominant species. Microscopists may also stop examining a blood film when the first parasite is found and identified. In general, successful detection of mixed infections usually requires longer and more careful study of thin blood films submitted for diagnosis.

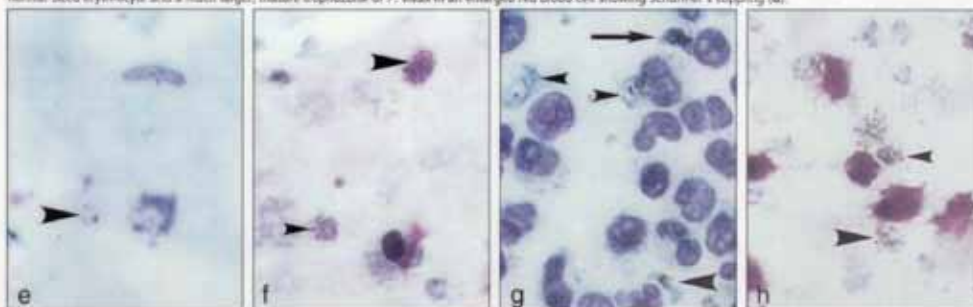
Thin and thick blood films are prepared from each patient on the same slide; the thick film should be examined first. Mixed infections will be found most easily in thick films because of the larger volume of blood examined. Microscopists who routinely examine thick blood films are more likely to detect low-density parasitaemias as well as have a better opportunity to find key diagnostic stages of each

malaria species present. When there is doubt about the presence of more than one species, multiple infections can be confirmed by careful evaluation of the thin film prepared on the same slide.

The most common mixed infection is probably *P. falciparum* with *P. vivax*. However, any combination is possible, depending on the geographical area. Although mixed infections with two plasmodial species are the most common, infections with three species are not uncommon. Mixed infections occur more commonly in children than in adults. As in the diagnosis of any malaria infection, species identification in mixed infections depends on recognition of the key diagnostic stages (e.g. schizonts or gametocytes in some cases), or parasite features (e.g. enlargement of erythrocytes, and presence or absence of stippling in some species). Ring forms (early trophozoites) of each species are typically distorted in thick blood films and are rarely useful in species determinations. The images below help to clarify these points.



Mixed infection in thin blood films: A sausage-shaped gametocyte of *P. falciparum* (a) and a young trophozoite (a, arrow) of *P. vivax* in an erythrocyte showing light Schüffner's stippling. A *P. falciparum* gametocyte with a mature *P. vivax* trophozoite in an enlarged red cell (b). A mature schizont of *P. vivax* with many merozoites and a large pigment mass at one side, and a smaller schizont of *P. malariae* containing fewer merozoites and only small pigment granules (c). A band-form trophozoite of *P. malariae* in a normal-sized erythrocyte and a much larger, mature trophozoite of *P. vivax* in an enlarged red blood cell showing Schüffner's stippling (d).














Mixed infections in thick blood films: A clearly visible, typical gametocyte of *P. falciparum* (e) with early (e, large arrow) and growing (e, small arrow) trophozoites of *P. vivax* adjacent to each other. A large, mature schizont of *P. vivax* (f, large arrow) demonstrating conspicuous pigment, and a typically smaller, mature schizont of *P. malariae* (f, small arrow). A triple infection (g), with a gametocyte of *P. falciparum* (g, large lower arrow), a mature schizont of *P. malariae* (g, large upper arrow) and two mature trophozoites of *P. vivax* (g, small arrows). A mixed infection of *P. vivax* and *P. malariae* with heavy parasitaemias of both (h); in the centre of the field, mature schizonts of *P. vivax* with many merozoites and conspicuous pigment, and a smaller *P. malariae* (h, small arrow) adjacent to it; a second schizont of *P. vivax* (h, large arrow) and trophozoites of both species scattered throughout the field.



Summary table

Same figure of old bench aids plate #8

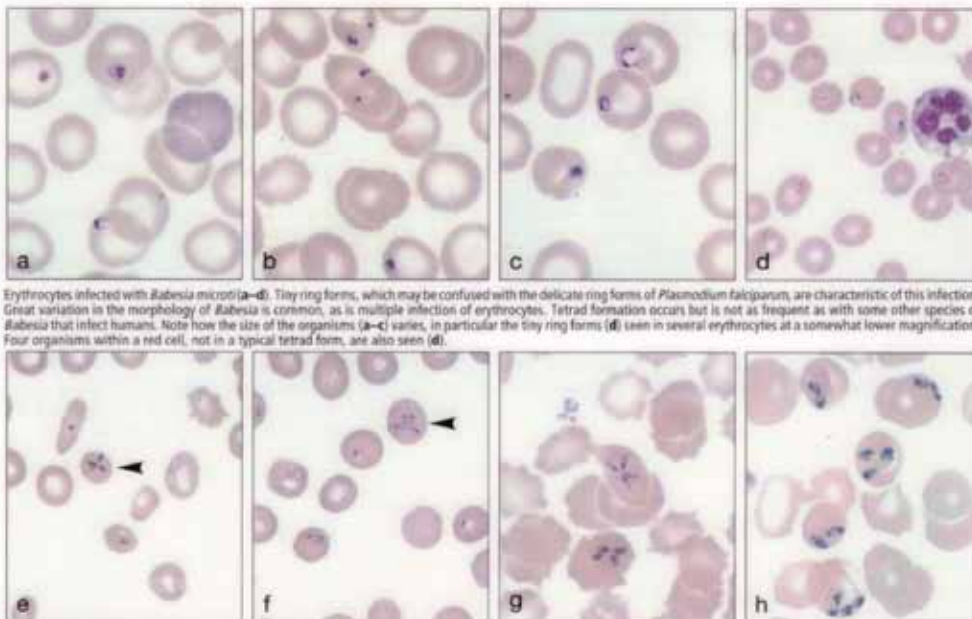
Species	Stage of parasite in peripheral blood		
	Trophozoite	Schizont	Gametocyte
<i>Plasmodium falciparum</i>	 <p>Size: small to medium; number: often numerous; shape: ring and comma forms common; chromatin: often two dots; cytoplasm: regular, fine to fleshy; mature forms: sometimes present in severe malaria, compact with pigment as few coarse grains or a mass.</p>	 <p>Usually associated with many young ring forms. Size: small, compact; number: few, uncommon, usually in severe malaria; mature forms: 12-30 or more merozoites in compact cluster; pigment: single dark mass.</p>	 <p>Immature pointed-end forms uncommon. Mature forms: banana-shaped or rounded; chromatin: single, well defined; pigment: scattered, coarse, rice-grain like; pink extrusion body sometimes present. Eroded forms with only chromatin and pigment often seen.</p>
<i>P. vivax</i>	 <p>Size: small to large; number: few to moderate; shape: broken ring to irregular forms common; chromatin: single, occasionally two; cytoplasm: irregular or fragmented; mature forms: compact, dense; pigment: scattered, fine.</p>	 <p>Size: large; number: few to moderate; mature forms: 12-24 merozoites, usually 16, in irregular cluster; pigment: loose mass.</p>	 <p>Immature forms difficult to distinguish from mature trophozoites. Mature forms: round, large; chromatin: single, well defined; pigment: scattered, fine. Eroded forms with scanty or no cytoplasm and only chromatin and pigment present.</p>
<i>P. ovale</i>	 <p>Size: may be smaller than <i>P. vivax</i>; number: usually few; shape: ring to rounded, compact forms; chromatin: single, prominent; cytoplasm: fairly regular, fleshy; pigment: scattered, coarse.</p>	 <p>Size: rather like <i>P. malariae</i>; number: few; mature forms: 4-12 merozoites, usually 8, in loose cluster; pigment: concentrated mass.</p>	 <p>Immature forms difficult to distinguish from mature trophozoites. Mature forms: round, may be smaller than <i>P. vivax</i>; chromatin: single, well defined; pigment: scattered, coarse. Eroded forms with only chromatin and pigment present.</p>
<i>P. malariae</i>	 <p>Size: small; number: usually few; shape: ring to rounded, compact forms; chromatin: single, large; cytoplasm: regular, dense; pigment: scattered, abundant, with yellow tinge in older forms.</p>	 <p>Size: small, compact; number: usually few; mature forms: 6-12 merozoites, usually 8, in loose cluster; some apparently without cytoplasm; pigment: concentrated.</p>	 <p>Immature and certain mature forms difficult to distinguish from mature trophozoites. Mature forms: round, compact; chromatin: single, well defined; pigment: scattered, coarse, may be peripherally distributed. Eroded forms with only chromatin and pigment present.</p>

Bench Aids for the diagnosis of malaria infections

Plate 11

Babesiosis

Human babesiosis (piroplasmosis) is an animal disease that can occasionally be transmitted to humans (i.e. a zoonosis) by the bites of infected ticks. *Babesia* spp. are intraerythrocytic protozoans belonging to the phylum Apicomplexa, which infect vertebrates throughout the world. The human infections include *B. microti*, a rodent parasite, *B. divergens*, a bovine parasite, and *B. equi* (reclassified as *Theileria equi*), an equine parasite. Like human malaras, *Babesia* spp. invade red blood cells; however, they do not produce pigments or cause enlargement of the host red blood cells. *Babesia* spp. differ from *Plasmodium* spp. in that they do not have an exoerythrocytic cycle, proper schizogony or gametocytes. Reproduction is by "budding" and produces only four merozoites, which are often arranged in a "Maltese Cross" pattern (e, arrow) or scattered at random in the red blood cells (h). Human cases have been recorded in Asia, Europe and North America, but not in Africa. Inside the host red blood cells the parasites are small (1–2.5 μm in diameter) and closely resemble the early ring forms of *P. falciparum*, with which they are most often confused. The diagnosis of *Babesia* spp. in blood films is therefore often difficult; certainly, many infections are incorrectly diagnosed as *Plasmodium* spp., especially as *P. falciparum*. *Babesia* spp. are rarely reported in humans; patients who have had their spleens removed are particularly at risk of symptomatic infection. Increased awareness of this disease may increase reporting, and microscopists should be alert to the possible presence of this infection.



Erythrocytes infected with *Babesia microti* (a–d). Tiny ring forms, which may be confused with the delicate ring forms of *Plasmodium falciparum*, are characteristic of this infection. Great variation in the morphology of *Babesia* is common, as is multiple infection of erythrocytes. Tetrad formation occurs but is not as frequent as with some other species of *Babesia* that infect humans. Note how the size of the organisms (a–c) varies, in particular the tiny ring forms (d) seen in several erythrocytes at a somewhat lower magnification. Four organisms within a red cell, not in a typical tetrad form, are also seen (d).

Typical tetrad forms of *Theileria equi* (formerly *Babesia equi*) are present in human blood films (e, f); other morphological forms of the parasite are visible in several other infected erythrocytes. An undescribed species of *Babesia* from California, USA, demonstrates tetrad formation in two erythrocytes (g). The pear-shaped organisms (h) in several red cells of a human blood film are similar in morphology to those of *Babesia divergens*.



Cleaning and storing microscope slides

Preparation

Slides for the preparation of blood films must be scrupulously clean and free from grease and moisture. Blood films made on dirty or greasy slides may wash off during the staining process. Scratched, "frozen" or iridescent slides should not be used for blood films (but can be used for other purposes in the laboratory).

Cleaning

New slides should be soaked in water with a reliable detergent for 30–60 minutes. Rinse slides under running tap water or in several changes of clean water. Dry each slide using a clean, lint-free cloth. Previously used slides must be soaked for at least 1 hour in hypochlorite solution before being washed. Soak these slides in water

containing detergent for 1–2 days. Using gauze or cotton wool, remove all traces of the old blood film and immersion oil. After cleaning, rinse slides under running tap water or in several changes of clean water. Dry each slide using a clean, lint-free cloth.

Storing

Clean slides (new or used) should be wrapped, in batches of 10, in thin paper secured with adhesive tape or rubber bands; slides can be stored and transported in cardboard slide boxes. In tropical and subtropical areas, clean slides should be stored in a dry place or in a warm-air cabinet. If stored at room temperature with high humidity, slides will stick together after a few weeks.

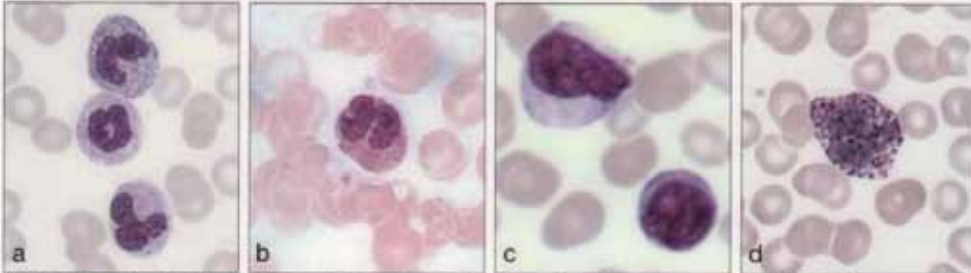
Care of the microscope

- **Do** always keep the microscope covered with a clean plastic or cloth cover when it is not in use to protect it from dust, especially in hot dry climates.
- **Do** protect the microscope from fungus growth in warm humid climates by one of the following: storing it in a continuously air-conditioned or dehumidified room; fixing a 15-watt bulb, which is left constantly lit, in the microscope box; or connecting a number of 15- or 25-watt bulbs, left constantly lit, inside a cupboard with tightly fitting doors.
- **Do** clean the immersion oil from the immersion objective every day.
- **Do** quote the model number and, if possible, the instrument and part number when ordering replacement parts.
- **Don't** dismantle the microscope to clean inaccessible parts.
- **Don't** use alcohol to clean the microscope.
- **Don't** clean the eyepieces with anything but dry lens tissue.
- **Don't** leave the lens ports empty; use the appropriate cover provided or close them with sealing tape.
- **Don't** exchange lenses or parts of the microscope.
- **Don't** store separate eyepieces and objectives without sealing each in an airtight plastic bag with a sachet of self-indicating silica gel. Self-indicating silica gel is blue when active and turns pink when it has absorbed all the water that it can. It can be reactivated by heating and will become blue again as this reactivation takes place.
- **Don't** store the microscope in its box for long periods or transport it without the retaining screw.

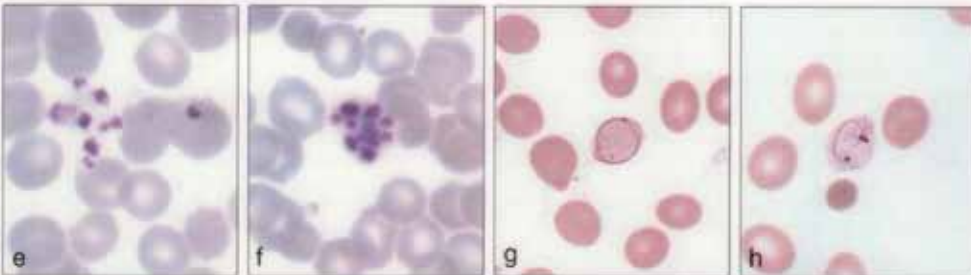
Bench Aids for the diagnosis of malaria infections

Plate 12

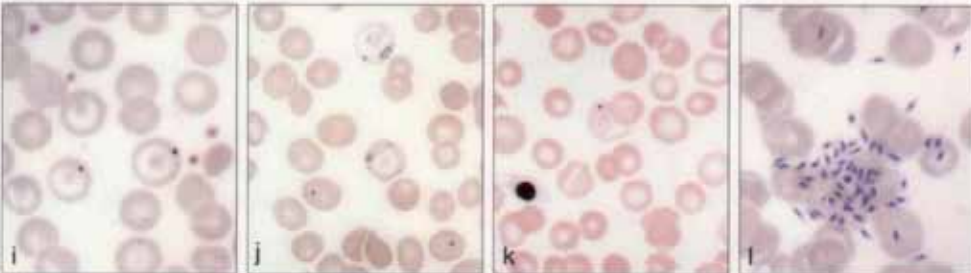
Artifacts and cellular elements in blood films



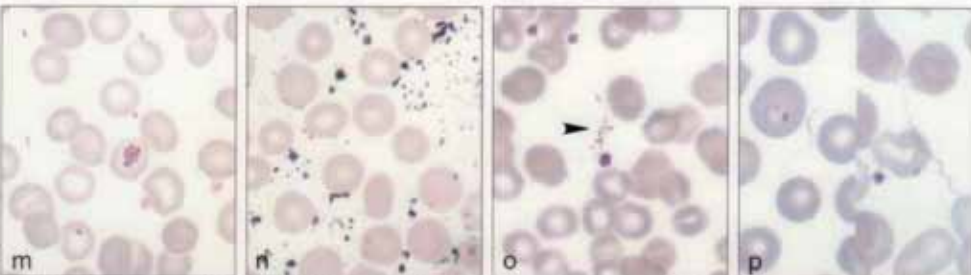
White blood cells (leukocytes) in blood films (a–d): neutrophils (a), an eosinophil (b), a lymphocyte (the smaller cell) and a monocyte (c), and a basophil (d) are commonly found when blood films are examined.



Platelets (e, f) lying free among red blood cells; when individual platelets are superimposed upon erythrocytes (e), or if they form a cluster (f), they superficially resemble schizonts and may be wrongly identified. Cabot's rings (g, h), a type of red cell inclusion that often takes the form of an oval ring, occur in severe anaemias and are thought to be remnants of spindle fibres forming during mitosis.



Howell-Jolly bodies (i, j) are purple-staining granules and represent nuclear (DNA) fragments. They are sometimes confused with the chromatin dots associated with malaria parasites. Pappenheimer bodies are small, irregular, basophilic deposits in erythrocytes; they may occur in association with Howell-Jolly bodies (j) or may be found alone in the erythrocyte (k). Other objects (l) that cannot be readily identified may also be confused with malaria parasites by inexperienced microscopists.



Bacterial organisms may contaminate blood films, and in this case a cluster of bacteria are superimposed on an erythrocyte (m). Blood films that are poorly rinsed following staining may retain the stain (n). In anticoagulated malarious blood kept at room temperature for several hours or more, microgametocytes may undergo exflagellation, releasing microgametes (o, arrow). In the split image (p), a single microgamete (note central nucleus) lies adjacent to a *P. vivax*-infected erythrocyte (left). On the right, a typical spirochaete of *Borrelia* is seen in a case of relapsing fever. Note the similarity to a malaria microgamete: the spirochaete is longer and thicker and has no nucleus.



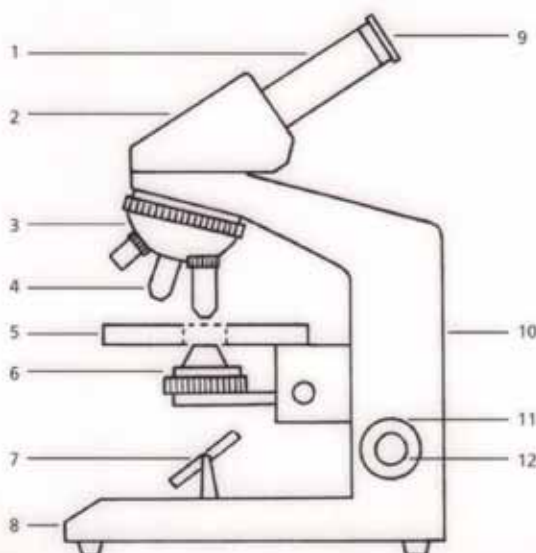
Use of the microscope

A lens combination of a x10 eyepiece and a x100 objective, to give a total magnification of x1000, is the standard used in most microscopes currently available. The microscope may have one eyepiece (monocular) or two (binocular). Binocular microscopes are easier and less tiring to use, particularly for long periods. All microscopes need a good source of light, either natural (daylight) or artificial (a microscope lamp powered by mains electricity, batteries or a generator). Most modern microscopes have a built-in lamp. Some microscopes have a detachable lamp that can be replaced with a mirror. Microscope lamps must also have a blue "daylight" filter to convert the "yellow" electric lamp light to "white" natural light. Daylight filters are used to minimize the differences in colour of stained malaria parasites in the blood film, which in turn makes diagnosis easier. The ability to control the intensity of the light source is essential for good microscopy, and most microscope lamps have an adjustable light output. All microscopes have a built-in substage condenser and iris diaphragm combination with which to achieve the final optimal light intensity. When a mirror is used with artificial light, the flat side should be used; when daylight is the light source, the concave side should be used without the substage condenser.

To set up the microscope, switch on the lamp or angle the mirror to reflect the light. Raise the substage condenser to its fullest extent and open the iris diaphragm to two-

thirds of its maximum aperture. Remove an eyepiece and look down the tube. If necessary, align the condenser/lamp or condenser/mirror to put the brightest light in the centre of the condenser. Replace the eyepiece. Raise the objective clear of the microscope stage. Place a drop of immersion oil on the rounded end of the thin blood film. In some countries anisole is used for work with the oil-immersion objective; this product has the same refractive index as immersion oil. Place the slide on the stage. Looking from the side of the microscope, lower the objective with the coarse adjustment until it is just touching the immersion oil. The film is now ready to be examined. Bring the microscopic field into focus using the fine adjustment. Adjust the light to a comfortable intensity. Use the same procedure to examine the thick blood film.

At the end of the work session the oil-immersion lens must be properly cleaned. If anisole is used, it evaporates from the blood film after some time, so that the film does not need to be cleaned and there is less chance of its being damaged. Use of anisole also means that the objective lens does not need to be cleaned. On the other hand, immersion oil must be gently removed with a lens paper slightly moistened with xylene. If examined blood films are to be kept they should be dipped in xylene, carefully wiped dry with tissue and placed in storage boxes.



Parts of a typical compound microscope

1. Main tube
2. Body tube (prism) } inclined head
3. Revolving nosepiece
4. Objective
5. Stage (mechanical stage)
6. Substage condenser with iris diaphragm
7. Mirror
8. Base (foot)
9. Ocular (eyepiece)
10. Arm (limb)
11. Coarse adjustment
12. Fine adjustment

Treatment of Parasitic Infections

The alternative drugs are listed in *italics*, infections are arranged alphabetically

INFECTION	DRUG OF CHOICE	ADULT DOSE	PEDIATRIC DOSE
Amebiasis (<i>E. histolytica</i>) Asymptomatic	Iodoquinol <i>Paromomycin</i> <i>Diloxanide furoate</i>	650 mg PO tid x 20d 25-35 mg/kg/d PO in 3 doses x 7d 500 mg PO tid x 10d	30-40 mg/kg/d (max. 2 g) PO in 3 doses x 20d 25-35 mg/kg/d PO in 3 doses x 7d 20 mg/kg/d PO in 3 doses x 10d
Mild to moderate intestinal disease	Metronidazole <i>Tinidazole</i>	500-750 mg PO tid x 7-10d 2 g once PO daily x 3d	35-50 mg/kg/d PO in 3 doses x 7-10d ≥3yrs: 50 mg/kg/d (max. 2 g) PO in 1 dose x 3d
Severe intestinal and extraintestinal disease (hepatic abscess)	Metronidazole <i>Tinidazole</i>	750 mg PO tid x 7-10d 2 g once PO daily x 5d	35-50 mg/kg/d PO in 3 doses x 7-10d ≥3yrs: 50 mg/kg/d (max. 2 g) PO in 1 dose x 3d
Ascariasis	Albendazole <i>Mebendazole</i>	400 mg PO once 100 mg PO bid x 3d or 500 mg once	400 mg PO once 100 mg PO bid x 3d or 500 mg once
Balantidiasis	Tetracycline <i>Metronidazole</i> <i>Iodoquinol</i>	500 mg PO qid x 10d 750 mg PO tid x 5d 650 mg PO tid x 20d	>8 yrs: 40 mg/kg/d (max. 2 g) PO in 4 doses x 10d 35-50 mg/kg/d in 3 doses x 5d 40 mg/kg/d in 3 doses x 20d
Blastocystosis	Metronidazole <i>Iodoquinol</i>	750 mg PO tid x 10d 650 mg PO tid x 20d	750 mg PO tid x 10d 650 mg PO tid x 20d
Capillariasis	Mebendazole <i>Albendazole</i>	200 mg PO bid x 20d 400 mg PO daily x 10d	200 mg PO bid x 20d 400 mg PO daily x 10d
Cryptosporidiosis	Nitazoxanide	500 mg PO bid x 3d	1-3yrs: 100 mg PO bid x 3d 4-11yrs: 200 mg PO bid x 3d >12yrs: 500 mg PO q12h x 3d
Cutaneous Larva Migrans	Albedazole <i>Ivermectin</i>	400 mg PO daily x 3d 200 mcg/kg PO daily x 1-2d	400 mg PO daily x 3d 200 mcg/kg PO daily x 1-2d
Cyclosporiasis	Trimethoprim- Sulfamethoxazole	TMP 160 mg/SMX 800 mg PO bid x 7-10d	TMP 5 mg/kg/d / SMX 25 mg/ kg/d PO in 2 doses x 7-10d
Cystoisosporiasis	Trimethoprim- Sulfamethoxazole	TMP 160 mg/SMX 800 mg PO bid x 10d	TMP 10 mg/kg/d / SMX 50 mg/ kg/d PO in 2 doses x 10d
Dientamoeba fragilis infection	Iodoquinol <i>Paromomycin</i> <i>Tetracycline</i> <i>Metronidazole</i>	650 mg PO tid x 20d 25-35 mg/kg/d PO in 3 doses x 7d 500 mg PO qid x 10d 500-750 mg PO tid x 10d	30-40 mg/kg/d (max. 2 g) PO in 3 doses x 20d 25-35 mg/kg/d PO in 3 doses x 7d >8yrs: 40 mg/kg/d (max. 2 g) PO in 4 doses x 10d 35-50 mg/kg/d PO in 3 doses x 10d
Malaria continued... Chloroquine-sensitive <i>P.</i> <i>vivax</i> , <i>P. ovale</i> , and <i>P.</i> <i>malariae</i>	Chloroquine (CQ)	1 g (600 mg base) PO, then 500 mg (300 mg base) 6 hrs later, then 500mg (300 mg base) at 24 hrs and 48 hrs	10 mg base/kg (max. 600 mg base) PO, then 5 mg base/kg 6 hrs later, then 5 mg base/kg at 24 hrs and 48 hrs
Chloroquine-resistant <i>P.</i> <i>vivax</i> , <i>P. ovale</i> , and <i>P.</i> <i>malariae</i>	Artemether- lumefantrine	20 mg / 120 mg tablet: 4 tablets given twice a day for 3 days	20 mg/120 mg tablet: 5-14 kg: 1 tablet 15-24 kg: 2 tablets 25-34 kg: 3 tablets >34 kg: 4 tablets given twice a day for 3 days

INFECTION	DRUG OF CHOICE	ADULT DOSE	PEDIATRIC DOSE
In pregnancy	Quinine-clindamycin <i>Artesunate-clindamycin</i>	600 mg / 150-300 mg q8h x 7d 4 mg/kg/day artesunate with 150-300 mg clindamycin q8h x 7d	
Chemoprophylaxis	Doxycycline CQ (in CQ-sensitive areas only)	100 mg/day, 2 days before and continuing for 4 wks after last exposure 5 mg/kg (8.3 mg salt/kg) weekly, 1 week before and continuing for 4 wks after last exposure	>8yrs: 2 mg/kg up to 100 mg/day, 2 days before and continuing for 4 wks after last exposure 5 mg/kg (8.3 mg salt/kg) weekly, 1 week before and continuing for 4 wks after last exposure
Microsporidiosis Ocular	Albendazole plus fumagillin	400 mg PO bid with fumagillin eye drops	
Intestinal <i>E. bineusi</i> <i>E. intestinalis</i>	Fumagilin Albendazole	20 mg PO tid x 14d 400 mg PO bid x 21d	
Disseminated	Albendazole	400 mg PO bid	
Parastrongyliasis (<i>Parastrongylus cantonensis</i>)	No drug is proven effective. Analgesic and corticosteroids can be used for symptomatic relief. Self-limited course with complete recovery. Mebendazole and glucocorticoids have been shown to shorten the course of infection.		
Scabies	Permethrin (5.0%) <i>Ivermectin</i>	Topically once, may repeat after 10-14 days 200 mcg/kg PO once	Topically once, may repeat after 10-14 days 200 mcg/kg PO once
Strongyloidiasis	Ivermectin <i>Albendazole</i>	200 mcg/kg/d PO x 2d 400 mg PO bid x 7d	200 mcg/kg/d PO x 2d 400 mg PO bid x 7d
Tapeworm infections Intestinal (<i>Taenia</i> spp., <i>Dipylidium</i> sp., <i>Diphyllobothrium</i> sp.) <i>Hymenolepis nana</i>	Praziquantel <i>Niclosamide</i> Praziquantel	5-10 mg/kg PO once 2 g PO once 25 mg/kg PO once	5-10 mg/kg PO once 50 mg/kg PO once 25 mg/kg PO once

Information on Some Anti-Parasitic Drugs

DRUG	ADVERSE EFFECTS	USE IN PREGNANCY AND BREASTFEEDING
Albendazole	Transient abdominal pain and diarrhea; tend to occur in patients being treated for heavy infection; headache and dizziness have been reported	Should not be administered during the first trimester or in suspected pregnancy; safe in later trimesters
Artemisinin-derivatives	Fever, cough, vomiting, and headache	Should not be administered during the first trimester
Chloroquine	Transient headaches and gastrointestinal distress; intolerance requiring withdrawal of treatment is rare; severe pruritus can occur; may precipitate a severe exacerbation of psoriasis; immediate adverse effects include nausea, vomiting, uneasiness, hypotension	No untoward effects demonstrated but treatment best deferred, when possible until after first trimester of pregnancy; chloroquine is excreted in breastmilk, so a decision must be made by the physician whether to discontinue breastfeeding or discontinue the drug
Diethylcarbamazine	Mazzotti-like reaction induced by disintegrating microfilariae and dead adult worms; immediate symptoms include fever, headache, dizziness, anorexia, malaise, urticaria, vomiting, and asthmatic attacks; effects usually subside by fifth treatment day; risk of meningoencephalitis if microfilaremia is heavy; proteinuria may occur	Should not be administered until after delivery; it is not known whether DEC is excreted in human breastmilk
Diloxanide furoate	Mild gastrointestinal symptoms, particularly flatulence; pruritus and urticaria may occur	No untoward effects have been demonstrated but treatment is best deferred until after the first trimester
Ivermectin	Mazzotti reaction is rarely severe but postural hypotension may occur in some patients; headache, pruritus, rash, arthralgia, myalgia, lymphadenopathy, lymphadenitis, edema, nausea, diarrhea, and vomiting within three days may occur; mild and require no more than simple reassurance	Should not be administered until after delivery; breastfeeding mothers should not be treated until the infant is at least 1 week old (by which time the blood-brain barrier should be fully developed)
Mebendazole	Transient abdominal pain and diarrhea; tend to occur in patients being treated for heavy infection; headache and dizziness have been reported	Should not be administered during the first trimester or in suspected pregnancy; safe in later trimesters
Mefloquine	Generally well tolerated; nausea, dizziness, disturbed sense of balance, vomiting, diarrhea, abdominal pain, and loss of appetite; neurological side effects include vertigo, blurred vision, and abnormal coordination; hallucinations, seizures, and	
psychosis have been reported	Evidence of embryotoxicity and teratogenicity in animal studies; avoid during first trimester; CDC recommends it as drug of choice for prophylaxis during pregnancy during second and third trimester; excreted in human breastmilk	

DRUG	ADVERSE EFFECTS	USE IN PREGNANCY AND BREASTFEEDING
Metronidazole	Generally well tolerated; headache, gastrointestinal irritation, and a persistent metallic taste are common; less frequently, drowsiness, rashes, and darkening of urine; serious and rare adverse effects (extended course treatment) include stomatitis, candidiasis, reversible leukopenia, and sensory peripheral neuropathy; alcohol may induce abdominal pain, vomiting, flushing, and headache	Should not be used in early pregnancy; breastfeeding should be interrupted until 24 hours after cessation of treatment
Praziquantel	Well tolerated in dosages recommended for intestinal tapeworms; occasionally causes abdominal discomfort, nausea, headache, dizziness, and drowsiness	Has not been shown to be mutagenic, teratogenic, or embryotoxic; delay breastfeeding during treatment and 72 hours thereafter
Primaquine phosphate	Gastrointestinal symptoms include anorexia, nausea, and abdominal pain; acute hemolytic anemia may occur in patients with G6PD deficiency	Contraindicated in pregnancy
Pyrantel pamoate	Mild gastrointestinal disturbance, headache, dizziness, drowsiness, insomnia, and rash	Should not be administered during first trimester of pregnancy
<i>Quinine dihydrochloride and quinine sulfate</i>	Serious reactions infrequent provided plasma concentration is not allowed to go above 15 mg/L; mild to moderate cinchonism: tinnitus, headache, blurred vision, altered auditory acuity, nausea, and diarrhea; pruritus, urticaria and erythematous rashes may occur; dysrhythmias, hypotension, and cardiac arrest are dose related	Quinine is safe in pregnancy. The risk of quinine-induced hypoglycemia is, however, greater than in non-pregnant women, particularly in severe disease. Special vigilance is therefore required.
Sulfadoxine pyrimethamine	Uncommon adverse effects include sulfonamide-induced hypersensitivity such as erythema multiforme and toxic epidermal necrolysis; hemolysis occurs in G6PD-deficient patients; adverse effects due to pyrimethamine are dose related and are reversible; includes anorexia, abdominal cramps, ataxia, tremors, and seizures	Generally safe during second and third trimesters; no clinical evidence that the use of sulfa drug-pyrimethamine combinations for malaria treatment in pregnant women has any effect on the fetus; there does not appear to be an increased risk of kernicterus; considered safe in breastfeeding
Tetracycline	Gastrointestinal irritation, teeth discoloration, and enamel hypoplasia (permanent), transient depression of bone growth, phototoxic reactions	Contraindicated in pregnancy, breastfeeding, and children below 8 years of age
Tinidazole	Metallic taste, nausea, vomiting, rash	Contraindicated in first trimester of pregnancy and lactation

List of More Recent National Policies and Guidelines on Parasitic Diseases

PARASITIC DISEASES	POLICIES/GUIDELINES
Food- and water-borne diseases	<ol style="list-style-type: none"> 1. DOH-Administrative Order 2006-0001 <i>Operational Guidelines for Parasitologic Screening of Food Handlers</i> 2. DOH-Administrative Order 2010-0037 <i>Diagnosis and Treatment Guidelines for Paragonimiasis</i> 3. DOH-Administrative Order 2009-0021 <i>Diagnosis and Treatment Guidelines for Capillariasis Infections</i>
Lymphatic filariasis	<ol style="list-style-type: none"> 1. DOH-Administrative Order 2010-0009 <i>Guidelines on the Prevention of Disabilities due to Lymphatic Filariasis</i> 2. Executive Order 369 s. 2004 <i>Establishing the National Program for Eliminating Lymphatic Filariasis and Declaring the Month of November of Every Year as Mass Treatment for Filariasis in Established Endemic Areas</i> 3. DOH-Administrative Order 2004-0157 <i>Declaring the Month of November of Every Year as the Mass Treatment Month for Filariasis in Established Endemic Areas in the Philippines</i> 4. DOH-Administrative Order 1998-0025A <i>The National Filariasis Control Program: Strategy Shift from Filariasis Control to the Elimination of Filariasis</i>
Malaria	<ol style="list-style-type: none"> 1. DOH-Administrative Order 2009-0001 <i>Revised Policy and Guidelines on the Diagnosis and Treatment of Malaria</i>
Schistosomiasis	<ol style="list-style-type: none"> 1. DOH-Administrative Order 2007-0015 <i>Revised Guidelines in the Management and Prevention of Schistosomiasis</i> 2. DOH-Administrative Order 2007-0015A <i>Amendments to Administrative Order 2007-0015</i>
Soil-transmitted helminthiasis	<ol style="list-style-type: none"> 1. DOH-Administrative Order 2010-0023 <i>Guidelines on Deworming Drug Administration and the Management of Adverse Events Following Deworming (AEFD)</i> 2. DOH-Administrative Order 2006-0028 <i>Strategic and Operational Framework for Establishing Integrated Helminth Control Program (IHCP)</i>
For further reading: http://home.doh.gov.ph/ao/ao_all.asp	

About the Author